

Study of Polymorphisms in the 5' Flanking Region of the Ovine IGF-I Gene in Zel Sheep

¹Mahmood Honarvar, ²Mostafa Sadeghi, ²Hossein Moradi-Shahrehabak, ²Shahab Behzadi,
²Hossein Mohammadi and ³Abolghasem Lavaei

¹Department of Animal Science, Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran

²Department of Animal Science, Faculty of Agricultural Science and Engineering,
University College of Agriculture and Natural Resources (UTCAN),
University of Tehran, Karaj, Iran

³Department of Animal Science, Islamic Azad University, Karaj Branch, Karaj, Iran

Abstract: The insulin-like growth factor 1 (IGF-I) gene has been described in several studies as a candidate gene for growth traits in farm animals. The objective of this study was to search for polymorphisms and gene regulatory sequences in the 5' flanking region of the Iranian sheep insulin-like growth factor I (IGF-I) gene. The DNA of 131 sheep of the indigenous Iranian Zel breed was evaluated. Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis of the 5' flanking region of the ovine IGF-I gene. PCR SSCP analysis of the 5' flanking region revealed four banding patterns. Family study indicated that these patterns in Zel breed sheep corresponded with four genotypes (with their frequencies in parentheses) A (0.37), B (0.41), C (0/02) and D (0.18).

Key words: Insulin-like growth factor I • Ovine • Single-strand conformation polymorphism • Zel.

INTRODUCTION

IGF-1 is a polypeptide of the molecular weight 7.5 kDa built of 70 amino acids [1]. The amino acid sequence of IGF-1 is identical in humans, cattle, dogs and pigs [2]. In humans the IGF-1 gene contains 6 exons and is about 90 kbp-long [2,3]. Due to an alternative splicing of exons 1 and 2, two different transcripts are formed: the one with exon 1 containing 1155 nucleotides (nt), while the other one, with exon 2, is shorter and contains 750 nt. Production of these transcripts is controlled by two different promoters both containing canonical regulatory sequences - TATA-box and CCAAT-box [4]. It was shown that transcripts of both classes are differentially expressed in various tissues, being, however, most abundant in liver [5]. Insulin-like growth factor I (IGF-I) is a polypeptide hormone that regulates growth and cellular metabolism during all stages of development in vertebrates. Most of the effects of growth hormone (GH) on somatic growth are mediated by IGF-I [6]. Due to its important roles in growth, IGF-I is an important candidate gene for growth traits in agricultural

animals. DNA polymorphisms at the IGF-I gene are effective genetic markers for growth traits in domestic animals [7]. Detection of single nucleotide polymorphisms is important because nucleotide substitutions in regions such as transcription factor binding sites in the genome may change the level of gene expression. Polymorphisms in the bovine IGF-I gene are associated with circulating IGF-I concentrations and growth traits [8]. Additional polymorphisms in growth hormone axis genes that are associated with production traits in ruminants have been reported [9]. The objectives of this study were to search for the same polymorphism in sheep that was found in cattle using the primers of Ge *et al.* [10] and to analyze the 5' flanking region of the sheep IGF-I gene for the presence of promoter sequences.

MATERIAL AND METHODS

Animals: Animals used in this study were Native Iranian sheep. We used 131 tailed Zel sheeps. This breed sheep are without fat-tail.

DNA Extraction and PCR Amplification of Sheep IGF-I gene:

For each sheep, 4 mL of total blood was collected from the left jugular vein using vacuum tubes containing 7.5 mg of EDTA and genomic DNA was purified from 1000- μ L of blood samples using the salting-out procedure of Miller *et al.* [11].

PCR was performed in a TECHNE thermocycler, model FTGRAD2D (TECHNE, England). A 265 bp fragment of the ovine IGF-I gene that was amplified. The IGF-I gene was amplified in a final volume of 25 μ L volume using 50 ng of genomic DNA, 1.25 μ L of each primer (0.5 pM), 0.8 mM of dNTPs, 1.5 mM of MgCl₂ and 0.06 U Taq polymerase (Gene net).

The thermal profile consisted of 3 min at 95°C, followed by 35 cycles of 45 s at 95°C, 40 s at 60°C and 50 s at 72°C, with a final extension of 10 min at 72°C.

The Primer Sequences Are as Follows:

F: 5'-ATTACAG CTGCCTGCCCCTT-3'

R: 5'-CACATCTGCTTACACCTTACCCG-3'

Amplicons were visualized by electrophoresis in 1% agarose gels, using 1x TBE buffer (89 mM Tris, 89 mM boric acid and 2 mM Na₂EDTA) containing 200 ng/ml of ethidium bromide.

Single-strand Conformational Polymorphism Analysis:

A 5- μ L aliquot of each amplicon was mixed with 12 μ L of loading dye (95% formamide, 0.05% bromphenol blue and 0.05% xylene cyanol). After denaturation at 95°C for 10 min, samples were rapidly cooled on wet ice and then loaded on 20cm \times 22 cm, 12% acrylamide: bisacrylamide (37.5:1) (Bio-Rad) gels. The gel was run at a constant voltage of 250 V at 20°C for 24 h. Gels were silver-stained according to the method of Sanguinetti *et al.* [12].

RESULTS AND DISCUSSION

A total of 131 sheep were genotyped for polymorphism in the IGF-I gene. The PCR amplified a 265 bp fragment from exon1 of the ovine IGF-I gene (Figure 1). After optimization of the parameters that affect the detection of SSCP's, we analyzed the PCR products from 131 animals. The PCR-SSCP analysis of exon1 IGF-I revealed four distinct patterns (Figure 2). The frequencies were 49% for pattern (1), 55% for pattern (2), 3% for pattern (3) and 24% for pattern (4). We observed most frequency in pattern of 2 and low frequency in pattern of 3. There are the great diversity of patterns which this is a race that may be due to specific genotypes.

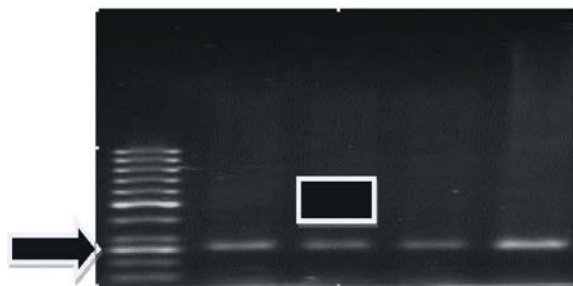


Fig. 1: PCR products analyzed by Electrophoresis in a 1.5% agarose gel with ethidium bromide staining

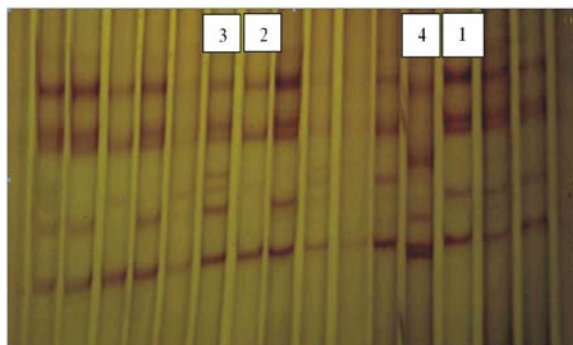


Fig. 2: PCR-single-strand conformational polymorphism of the ovine IGF-I gene. three SSCP patterns were detected with electrophoresis conditions: 250V, 20°C for 24h.

IGF-I has a remarkable diversity of biological effects. It is well known that IGF-I plays an important role both in embryonic and postnatal growth. Circulating IGF-I concentrations correlate with fetal and neonatal size in several species [13, 14]. IGF-I promotes growth of fetal organs, endocrine gland and skeletal maturation in fetal sheep [15], in part by enhancing fetal amino acid and glucose uptake [15, 16]. In postnatal life, IGF-I is a key determinant factor in linear growth of animals, 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) [17-19]. The polymorphism in question was first reported in Angus cattle by Ge *et al* [10] as SSCP. This polymorphism was then identified as T/C transition, also recognizable as RFLP-SnaBI [8]. 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, we observed three conformational patterns. Yilmaz *et al* [20] identified two SNPs in this region of the sheep IGF-I gene, which were a T-C transition and a G-C transversion. Therefore, further study in other breeds is needed to confirm our result.

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