Insulin-like growth factor I (IGF-I) is an important growth factor involved in a variety of physiological processes including reproduction, fetal development, and growth (Adam et al. 2000; Shen et al. 2003). 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 (Ge et al. 2001). Additional polymorphisms in growth hormone axis genes that are associated with production traits in ruminants have been reported (Grochowska et al. 2001). 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. (1997) and to analyze the 5’ flanking region of the sheep IGF-I gene for the presence of promoter sequences.

Genomic DNA was obtained using blood samples collected from 46 sheep located at the Ohio Agricultural Research and Development Center’s Wooster campus. Animals used in this study were purebred Polypays and crossbreds consisting of the Hampshire, Targhee, Rambioullet, Dorset and Suffolk breeds. Although not as common as some other breeds of sheep, Polypay is still an important breed in the United States that combines superior reproductive performance of Finnsheep, adaptability and quality fleeces of Rambouillet,
Sequences of primers that were used in PCR-SSCP analysis were reported previously (Ge et al. 1997). The fragments amplified using DNA from homozygous individuals, as determined by PCR-SSCP, were sequenced and aligned next to each other. Two single-nucleotide polymorphisms were identified. IGFF and IGFR primers were designed to perform restriction fragment length polymorphism (RFLP) analysis. Sequences of IGFF and IGFR were 5’ GGGGCAGGCA GCTTTGTAAT-3’ and 5’ TCACATCTGCTA ATACACCTTACCCG-3’, respectively.

PCR cloning was conducted for the amplified fragments using a pGEM-T easy cloning vector (Promega, Madison, WI). The DNA band was purified from the agarose gel using the Nucleotrap gel purification kit (Clontech, Palo Alto, CA). Insertion of the PCR fragments into the vector was carried out at 4°C overnight, and transformation was conducted with JM109 competent cells (Promega, Madison, WI). The plasmid DNA was purified using the Mini-plasmid prep kit (Qiagen, Valencia, CA). Sequencing was conducted with an ABI377 machine at the Molecular Cellular Imaging Center of the Ohio Agricultural Research and Development Center (Wooster, OH).

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RFLP analysis was performed by incubating a mixture of 4 μL of PCR product, 4.6 μL of distilled water, 1 μL of enzyme buffer, and 0.3 μL of enzyme at 37°C for 3 h. Gels were visualized using a 4% agarose gel that contained ethidium bromide.

A fragment of 265 bp in the 5’ flanking region of the ovine IGF-I gene, 467 to 732 bp upstream from the 5’ end of Exon 1 (Wong et al. 1989; GenBank Accession Number: X17229), was amplified by PCR for SSCP analysis. Evaluation of SSCP results revealed three banding patterns. Family study in mixed breed sheep indicated that these patterns corresponded with three genotypes of (with their frequencies in parentheses) AA (0.70), AB (0.25), and BB (0.05), which arose from a one-locus, two allele (A, B) polymorphism. Genotypic frequencies in 22 purebred Polypay sheep were AA (0.77) and AB (0.23). Frequency of allele A in Polypays was 0.89. No deviation from Hardy-Weinberg equilibrium was observed in this study.

Fragments amplified using DNA from homozygous individuals were sequenced and aligned next to each other. Two single-nucleotide polymorphisms were identified. A T to C transversion was found at positions 179 and 181, respectively, of the amplified PCR product, resulting in recognition sites for Bsp143II and HaeI (Fermentas Inc., Hanover, MD). The amplified PCR products were digested separately with these enzymes.

To further investigate the nature of the polymorphisms detected, we assembled two sheep EST sequences (X69472 and X17229) along with our sequence (AF492765) into a single DNA fragment that spanned 2,162 bp upstream of...
the 5’ end of Exon 1 of the ovine IGF-I gene (Wong et al. 1989). Analysis of that fragment with a neural network promoter prediction program (Reese 2001) revealed a promoter sequence (in parentheses) located approximately 100 bp downstream of the two nucleotide substitutions detected in this study (GGCTCTGGAATAAAAATTTCTC GCCCATCCTCCACGAATATTTCTTCA; prediction score: 0.96). A second sheep promoter sequence was located at 1,690 bp upstream of the 5’ end of Exon 1 (TTTTCATATTTTTAAGCG CATCCACACGTTGCCAGTGCTTTTCTC; prediction score: 0.99). The software used in this study to detect sheep promoters has been successfully used to detect human promoters (Reese 2000) and a high degree of nucleotide sequence homology exists between human and sheep IGF-I 5’ flanking regions (this study). Therefore, the software can be used to detect sheep promoters. A fragment of 2,162 nucleotides upstream of Exon 1 in the human IGF-I gene was also retrieved from the human genome sequence released in May 2004. A 70% sequence homology was observed between the human and sheep DNA fragments. A human promoter was located only 64 bp downstream of the position of the second sheep promoter (i.e., 1,626 bp upstream of Exon 1), indicating that the location of that promoter is approximately conserved between the two species. Therefore, the nucleotide sequence, as well as the location of the promoter sequences, in human and sheep are similar.

The IGF-I alleles detected using SSCP analysis exhibited Mendelian inheritance and codominant segregation in six paternal half-sib progeny. The single nucleotide polymorphisms detected in this study should also follow codominant segregation, because we detected patterns consistent with the existence of two alleles during the SSCP analysis, confirmed the presence of these polymorphisms using RFLP, and found by family study that the progeny received only one allele from each of the parents.

REFERENCES


