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Non-Carrier Identification of Spider Lamb Syndrome in Iranian Baluchi and Karakul Sheep by PCR-RFLP

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Abstract: The aim of this study was to identify the Spider Lamb Syndrome (SLS) in Iranian Baluchi and Karakul Rams by PCR RFLP method. In total 200 blood samples were prepared from Baluchi and Karakul Rams from Abbas Abad and Karakul Sarakhs Animal Breeding Centers in Khorasan state of Iran. Genomic PCR RFLP protocol was performed to amplify the polymorphic region of the ovine Fibroblast Growth Factor Receptor 3 (FGFR3) gene from exon 17 of ovine Chromosome 6. PCR products from the polymorphic region of (FGFR3) gene (432 bp) were digested with *Btg*I enzyme. The results of this study demonstrated that there was no carrier of SLS in Iranian Baluchi and Karakul Rams in these Animal Breeding Centers.

Key words: SLS, FGFR3, PCR-RFLP, Iranian sheep

INTRODUCTION

Understanding the molecular basis of a genetic defect renders it possible to detect carriers directly at the DNA level and, what is more important, early in the animal's life and even in embryonic cells (Harlizius *et al*., 1996). The objective of the present study is identify the carrier of Spider Lamb Syndrome from Abbas Abad and Karakul Sarakhs Animal Breeding Centers in Khorasan state of Iran. One of the disorder in sheep breeding is Spider Lamb Syndrome (SLS), or hereditary chondrodysplasia, is a congenital osteopathy first described in young lambs during the early 1970s (Cockett *et al*., 1999) and was primarily observed in United States black-faced Suffolk and Hampshire sheep (Drogemuller *et al*., 2005). Several skeletal abnormalities are associated with the syndrome, including disproportionately long, spider-like legs, curvature of the spine, deformed ribs and sterna, facial deformities, lack of body fat and muscular atrophy (Cockett *et al*., 1999). Clinical presentation of the syndrome is also highly variable, with some lambs severely affected at birth and others developing the condition at 3-4 weeks of age (Rook *et al*., 1988). In contrast to SLS, naturally occurring gain-of-function mutations in FGFR3 cause various dwarfism phenotypes in humans, including achondroplasia, hypochondroplasia and thanatophoric dysplasia, all of which are inherited in a dominant manner (Ornitz and Marie, 2002). Because endochondral ossification and long-bone growth are polygenic processes, epistatic interactions are expected to influence skeletal growth.

FGFR3 is just one of the many important factors involved in the signalling pathways controlling bone growth (L'Hôte and Knowles, 2005); constitutive activation of MEK1 was recently shown to inhibit skeletal overgrowth in Fgfr3-null mice (Murakami *et al*., 2004). Fibroblast growth factor receptor 3 (FGFR3), a member of the tyrosine kinase II receptor family, functions to restrict the proliferation of pre-bone cartilage at the physes of long bones, thereby limiting skeletal elongation. FGFR3 functions as a negative regulator of cell proliferation and differentiation in growth plate chondrocytes (Smith *et al*., 2006) and it is phosphorylated upon ligand binding and inhibits chondrocyte proliferation through signal transducer and activator of transcription (STAT)-mediated induction of cell cycle inhibitors (Xiao *et al*., 2004). In this disorder, a T>A transversion at nucleotide position 69 of ovine FGFR3 exon 17 from Chromosome 6 causes a non-conservative amino acid substitution of non-polar valine (V) to charged glutamate (E) at residue 700 in the second tyrosine kinase domain of the receptor. It is possible that the mutation leads to loss of receptor function in homozygotes, which results in poorly controlled chondrocyte differentiation (Drogemuller *et al*., 2005). Deficiency of FGFR3 is a strong candidate for SLS because the physical and histological lesions characteristic of SLS-affected lambs are indicative of FGFR3 involvement in long-bone growth. Thus, the objective of the present study is identify the carrier of

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Spider Lamb Syndrome from Abbas Abad and Karakul Sarakhs Animal Breeding Centers in Khorasan state of Iran.

MATERIALS AND METHODS

For this study, in total 200 blood samples were prepared from Baluchi (n = 100) and Karakul (n = 100) Rams from Abbas Abad and Karakul Sarakhs Animal Breeding Centers in 2007. Genomic DNA was extracted from 100 µL of blood by Guanidium-Thiucianate Silica gel method (Boom *et al*., 1990). PCR reaction was performed for amplification of polymorphic region of the (FGFR3) gene. The following primers were used for PCR for (FGFR3) gene (Drogemuller *et al*., 2005).

F: 5-CCT TGT TTG ACC GCG TCT AC-3
R: 5-ATG TAC CTG GGG GAC ATG C-3

PCR was done in BiometraT-Personal Ver: 1.11 thermocycler by Gene pak PCR MasterMix Core Kit for amplification in a total volume of 20 µL. The PCR mix contained: 2.5 µL PCR buffer 10X, 2.5 mM $MgCl_2$, 2 mM dNTPs, 2 µL mix of oligonucleotids (10 pM from each primer), 1u Taq DNA polymerase and 7 µL ddH_2O. Electrophoresis was carried out on agarose gel 1% ethidium bromide and pBR322/*Alu*I was used as ladder (Fig. 1).

PCR products from polymorphic region of (FGFR3) gene (432 bp) were digested with *Btg*I enzyme at 65°C for 16 h. After digestion with *Btg*I, the T (wild-type) allele was represented by fragments of 63, 166 and 203 bp, whereas the A (mutated) allele was represented by fragments of 63 and 369 bp. Lengths of fragments (bp) of SLS PCR product after digestion with *Btg*I were 63, 166, 203 bp for animal which are free of SLS and 63, 166, 203, 369 for SLS Carrier genotypes. Digestion products were separated by electrophoresis on 8% acrylamid gel and visualized with silver staining method and M50 was used as ladder (Fig. 2).

RESULTS AND DISCUSSION

There was a sharp increase in reported cases of SLS in the early and mid-1980s (Cockett *et al*., 1999). Although many breeders have actively culled all suspected carriers

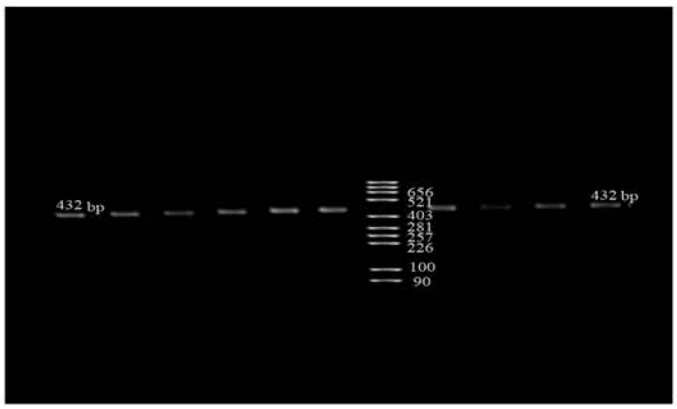

Fig. 1: Agarose gel electrophoresis of digestion products from FGFR3 gene reveled same band all lanes (432 bp). DNA Ladder is pBR322/*Alu*I

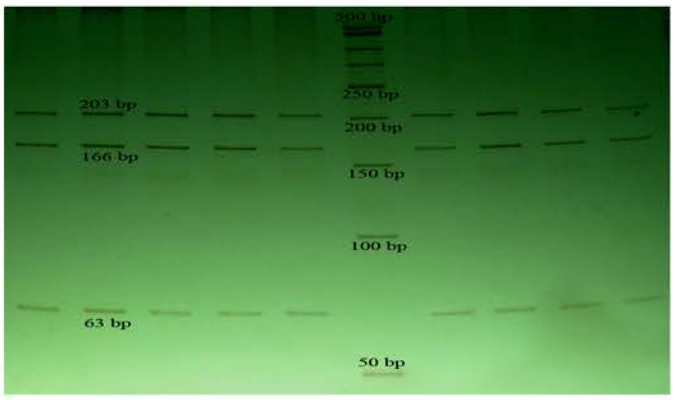

Fig. 2: Acrylamid gel electrophoresis of digestion products with *Btg*I. DNA Ladder is M 50

from their flocks, the frequency of the disorder remains relatively high. Traditional breeding methods such as progeny testing of potential breeding rams would reduce the frequency, but are time consuming and costly. Thus, the identification of genetic disorders at the DNA level seems necessary. In 1997, a genetic test for SLS, based on the A>T polymorphism in exon 17 of ovine FGFR3, was introduced to the sheep industry and later patented (Beever and Cockett, 2001). During the 7 years since the introduction of this test, the incidence of SLS has dramatically decreased. In New Zealand, above 2500 Suffolk sheep have been tested for SLS using the T>A SNP and there has been a subsequent reduction in the number of heterozygous individuals in stud flocks in this country (Jolly *et al*., 2004). A similar reduction in heterozygous animals has also been observed in the USA (Beever *et al*., 2006). During the height of the SLS crisis, 336 animals tested for SLS from five black-faced breeds. These animals had been entered in the show because they were thought to be some of the most outstanding breed representatives. Of these 336 animals, 97 (28.9%) individuals were carriers of SLS (Beever *et al*., 2006). In other study Genetic testing confirmed for SLS in Suffolk families in Germany. A total of 125 healthy unrelated Suffolk rams have been analysed and 11 SLS carriers were observed, indicating the frequency of the mutated FGFR3 allele is 4% (Drogemuller *et al*., 2005). In this study, there was no carrier of SLS in Iranian Baluchi and Karakul rams from Abbas Abad and Karakul Sarakhs Animal Breeding Centers in Khorasan state of Iran. However we did not observe any carrier for this genetic disease but it is safe to say that the rate of infectious amount Iranian endemic livestock is very low. Besides omitting all the infected lambs in the past few years at this center has led to a non-carrier rate. We can say that this disease and its source are from industrial livestock in Europe, not from Iranian endemic breeds, therefore from the fact that no carrier has been observed in this center does not follow that the disease does not exists in Iranian endemic sheep.

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REFERENCES

Beever, J.E. and N.E. Cockett, 2001. Screening for the molecular defect causing spider lamb syndrome in sheep. US Patent No., 6: 306-591 B1.

Beever, J.E., M.A. Smit, S.N. Meyers, T.S. Hadfield, C. Bottema, J. Albretsen and N.E. Cockett, 2006. A single-base change in the tyrosine kinase II domain of ovine FGFR3 causes hereditary chondrodysplasia in sheep. Anim. Genet., 37 (1): 66-71.

Boom, R., C.J.A. Sol, M.M.M. Salimans, C.J. Jansen, P.M. E. Wertheim-Van Dillen and J. Van Der Noordaa, 1990. Rapid and simple method for purification nucleic acid. J. Clin. Microbiol., 28 (3): 495-503.

Cockett, N.E., T.L. Shay, J.E. Beever, D. Nielsen, J. Albretsen, M. Georges, K. Peterson and A. Stephens, 1999. Localization of the locus causing spider lamb syndrome to the distal end of ovine chromosome 6. Mamm. Genome, 10 (1): 35-38.

Drogemuller, C., A. Wohlke and O. Distl, 2005. Spider Lamb Syndrome (SLS) mutation frequency in German Suffolk sheep. Anim. Genet., 36 (6): 511-542.

Harlizius, B., S. Schrober, I. Tammen and D. Simon, 1996. Isolation of the bovine uridine monophosphate synthase gene to identify the molecular basis of DUMPS in cattle. J. Anim. Breed. Genet., 133: 303-309.

Jolly, R.D., H.T. Blair and A.C. Johnstone, 2004. Genetic disorders of sheep in New Zealand: A review and perspective. N. Z. J. Vet., 52 (2): 52-64.

L'Hôte, C.G.M. and M.A. Knowles, 2005. Cell responses to FGFR3 signalling: Growth, differentiation and apoptosis. Exp. Cell Res., 304 (2): 417-431.

Murakami, S., G. Balmes, S. McKinney, Z. Zhang, D. Givol and B. de Crombrugghe, 2004. Constitutive activation of MEK1 in chondrocytes causes Stat1-independent achondroplasia-like dwarfism and rescues the Fgfr3-deficient mouse phenotype. Genes Dev., 18 (3): 290-305.

Ornitz, D.M. and P.J. Marie, 2002. FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. Genes Dev., 16 (12): 1446-1465.

Rook, J.S., M. Kopcha, K. Spaulding, P. Coe and M. Benson, 1988. Diagnosis of hereditary chondrodysplasia (spider lamb syndrome) in sheep. J. Am. Vet. Med. Assoc., 193 (6): 713-718.

Smith, L.B., M.R. Dally, R.D. Sainz, K.L. Rodrigue and A.M. Oberbauer, 2006. Enhanced skeletal growth of sheep heterozygous for an inactivated fibroblast growth factor receptor 3. J. Anim. Sci., 84 (11): 2942-2949.

Xiao, L., T. Naganawa, E. Obugunde, G. Gronowicz, D.M. Ornitz, J.D. Coffin and M.M. Hurley, 2004. STAT1 controls postnatal bone formation by regulating fibroblast growth factor signaling in osteoblasts. J. Biol. Chem., 279 (26): 27743-27752.