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Short Communication

CHARACTERISTIC FEATURES OF IFN-α GENE IN BEETAL GOAT BREED OF PUNJAB, PAKISTAN

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ABSTRACT

The immune systems of species are basically dynamic for evolution and survival, and the animal selection patterns in innate immune loci are of prodigious importance in molecular evolutionary research. Interferon alpha (IFN-α) plays a fundamental role in innate immune response with biological and antiviral activity. In the current study, the IFN-α gene was genetically and phylogenetically characterized in thirty Beetal goats by direct sequencing. Genomic DNA was isolated and sequencing analysis of IFN-α gene revealed two missense substitutions (A83G: Lys28Arg, A127G: Thr43Ala) and one synonymous substitution (A144G). Phylogenetic analysis and genetic distance showed the species-wise clustering and higher genetic distance with other goats. This information in Pakistani indigenous goats may provide useful insights into the design of molecular selection strategies for genetic improvement of animal and further biological studies in other goat breeds may provide evolutionary way to acquire better disease resistant animals.

Keywords: Interferon alpha, polymorphism, phylogenetic analysis, Beetal, Pakistan.

INTRODUCTION

Pakistan is rich in live-stock genetic resources, and they play important role in the economy of the country. Evolutionary studies in immune system genes are enormously important to show the evolutionary pattern and selection signatures in animals (Okpeku *et al.,* 2016). The immune system genes variations impact various traits, especially the animal's susceptibility to infectious agent and the autoimmune disease. In molecular evolutionary research, the animal selection patterns in innate immune loci are of great importance. Understanding the genetic evolution of immune system genes and insights in animal evolution and survival is useful approach in animal breeding and genetic improvement.

To the best of our knowledge it is preliminary work of genetic characterizing of immune system genes in Pakistani goat breed. Beetal goat is considered the superior animal among other goat breeds for better productive performance in the form of milk and meat production. Exploring animal's genome is of great importance in selection of highly heritable traits (Wajid *et al.,* 2013). In the current study, we had investigated the polymorphism and phylogenetic analysis of IFN-α gene in Pakistani indigenous Beetal goat breed. The results would be useful in understanding the significance of immune-diversity level in goat species in order to design molecular selection strategies for better disease resistant animals.

MATERIALS AND METHODS

Animals and Sample Collection: A total of 30 unrelated Beetal goats were selected with specific phenotypic features from Small Ruminants Research & Development Center, Rakh Khairewala, Layyah and Small Ruminant Training and Research Center, UVAS, Pattoki for the current study. Ten mL blood from the Jugular vein of Beetal goat was aseptically collected in a sterile falcon tubes of 50 mL having 200 µL Ethylenediamine tetra-acetic acid (0.5 M EDTA) as an anticoagulant. Important information related to breed, age, sex, animal ID and location was maintained as record. Blood samples were immediately placed on ice and brought to the molecular biology Lab of University of Veterinary and Animal Sciences, Lahore, Pakistan in order to store them temporarily at -20 °C in freezer before DNA extraction.

Nucleic acid Extraction and Gene Amplification: Genomic DNA was extracted from the whole blood using the method previously used by Hussain *et al.,* (2013). DNA samples were diluted in low TE buffer (pH 8.0) and total DNA was run through 0.8% agarose gel electrophoresis to examine their quality and quantified by

using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Pittsburg, PA, USA). All the DNA samples were brought to equal concentration of 50 ng/µL. All the samples were stored at -20 ˚C for further use. A primer pair IFNα-F: AAAGCATCTGCAAGGTCCCCGAT; IFNα-R: TCCTCCTGCGTCAGACAGGCTT was designed for the amplification of Interferon alpha (IFN-α) gene 361 base pair (bp). Amplification was performed using high fidelity platinum ®supermix PCR kit (Invitrogen, Carlsbad, CA, USA). C1000 thermal cycler (Bio-Rad, USA) was used for the amplification of IFN- α gene. Condition for PCR of IFN- α was: 95˚C for 5 min, followed by 30 cycles at 95˚C for 30 sec, 56˚C for 30 sec and 72˚C for 45 sec, with a final extension step at 72˚C for 10 min. The PCR products were analyzed by electrophoresis in 2% agarose gel, were purified and precipitated using 80% ethanol and the pelleted DNA was diluted in DEPC water.

Gene Sequencing and Analysis: The purified amplicons were sequenced using fluorescent dideoxynucleotide terminators in an ABI PRISM 3130 automated sequencer (Applied Biosystem, Inc, Foster City, CA). The nucleotide sequences assembly and editing was performed using BioEdit software v 7.2.5 (Hall, 1999). MEGA software (MEGA v6) was used for sequences variation analysis with sequences available at GenBank and phylogenetic analysis. The phylogenetic tree was inferred by using Maximum Likelihood method with reliability of the branching by using 1000 bootstrap replications (Tamura *et al*., 2013). The sequences were BLAST and further analyzed through multiple sequence alignment of IFN-α of Beetal goat with other species like goat (*Capra hircus*: GenBank accession number XM005683621), sheep (*Ovis aries*: X59067), cattle (*Bos taurus*: NP001165512), buffalo (*Bubalus bubalis*: XP006055575), Yak (*Bos grunniens*: AEU17776) chiru (*Pantholops hodgsonii*: XM005962133), and blackbuck (*Antilope cervicapra*: ACR61636).

RESULTS AND DISCUSSION

Beetal goat is a well-adapted animal, distributed over a vast region of Pakistan and India. Although the Beetal represents an important good milk producing breed, the implementation of appropriate management policy and improving the animal's health is needed to enhance its productive efficiency. Breeding animals resistant to disease are economically feasible and desirable. Genes involved in animal innate immune response predictably affect the clinical course of infection (Yakubu *et al.,* 2016). In molecular evolutionary research, exploring animal's genome in immunity level is of special interest in order to improve animal's health, fundamentally indispensable for evolution and survival of animal (Detilleux, 2011). This study was conducted to investigate the genetic variation in IFN-α gene in Beetal goat and compare with other caprine and ovine sequences available at GenBank. The size of the amplified PCR product was 361 nt and confirmed by direct sequencing. Of the examined IFN-α in thirty unrelated Beetal goat, we detected three transitional substitutions A83G, A127G and A144G (Table 1), (Figure 1A, 1B, 1C). The analysis showed two of them were missense substitution Lys28Arg and Thr43Ala and one was synonymous (amino acid remained unchanged at position 48). Alignment of partial IFN-α sequence of Beetal goat with other animals showed maximum homology with goat (98.33%), followed by sheep (95.11%), buffalo (94%), cattle (94%), yak (92%), chiru (89.17%), and blackbuck (86.33%).

The genetic relationship of partial sequences of IFN-α in Beetal goat and other published sequences of caprine, ovine and bovine was revealed by the phylogenetic analysis (Figure 2). The phylogenetic tree indicated the similar pattern of phylogenetic relationships to the trees constructed through mitochondrial sequences among the caprine, ovine, bovine and other animals as the small ruminants formed a single cluster indicating the close resemblance.

This is preliminary report of sequence variation of the IFN-α gene for any Pakistani goat breed. The polymorphic sites obtained in IFN-α gene of Beetal goat that may assist in the exploration of association with disease resistance in these animals. This genetic information is the baseline information; however, further molecular studies should be conducted involving other goat breeds to explore the potential application of immunity genes in genetic improvement efforts.

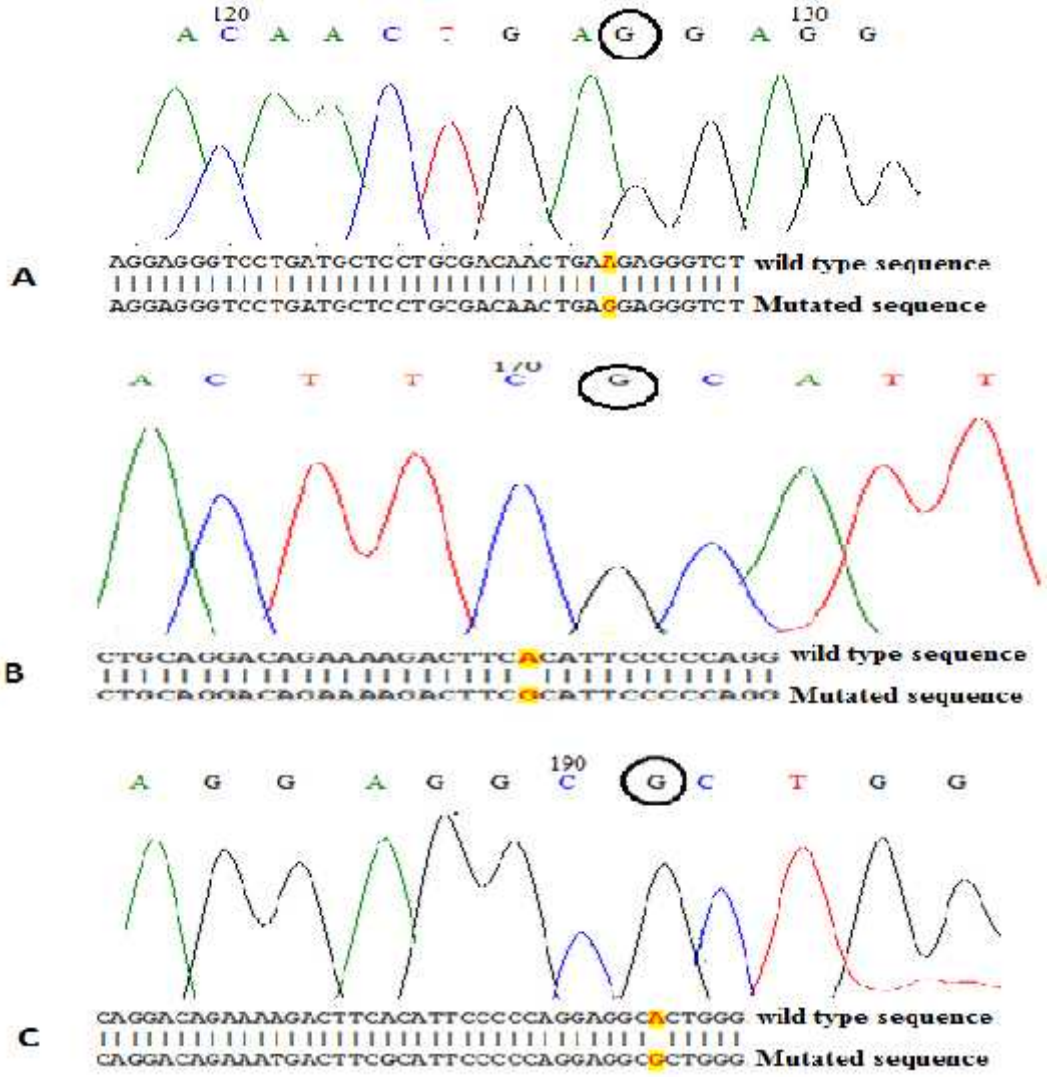

Figure 1. Chromatogram of substitutions A>G (A), A>G (B), A>G (C) in the Interferon alpha (IFN-α) gene of Beetal goat.

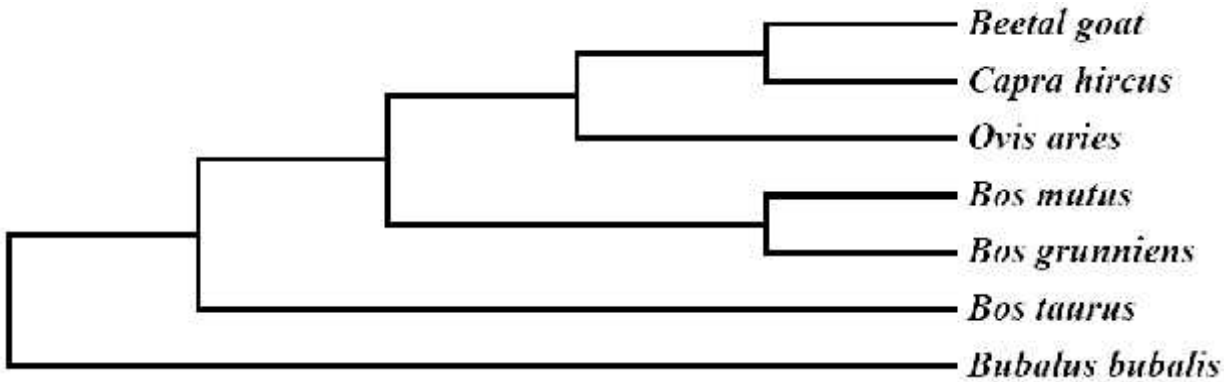

Figure 2: Neighbor-Joining based phylogenetic analysis was carried on the basis of partial IFN-α gene sequences of Beetal goat with other relative species.

Table 1. Polymorphic sites detected in the IFN-α region of Beetal Goat

Nucleotide Position	Wild type	Mutated	Transition/ Transversion
83	A	G	Transitional
127	A	G	Transitional
144	A	G	Transitional

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