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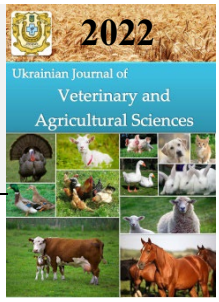

Molecular Phylogeny and Genetic Diversity of Interferon-α-A Domestic Yaks (*Bos grunniens*) in Pakistan

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Abstract

Among the livestock species, wild yaks (*Bos grunniens*) survive only in the Tibetan region and Pakistan K2 region. They have declined dramatically in range and numbers and, therefore, were domesticated even in Pakistan due to the multipurpose function of the animal, which provides meat, milk, and other dairy products. Interferons were initially characterized for their ability to `interfere' with viral replication, slow cell proliferation, and profoundly alter immunity. They are a group of hormone-like molecules synthesized and secreted by macrophages, monocytes, T lymphocytes, glia, and neurons. For this purpose, Domestic yak blood 53 samples were collected from Gilgit and Bultistan and genotyped using a set of labeled microsatellite loci for this purpose. After PCR, DNA fragment sizes were determined in an ABI 3130 Genetic Analyzer. All microsatellite markers were successfully amplified and exhibited a polymorphic nature. Phylogenetic analysis based on the microsatellite DNA IFN-α-A of the control region (401 bp) showed nine microsatellite DNA haplogroups identified in Pakistani domestic Yaks. Phylogenetic analysis of the IFN-α-A gene suggested the domestic yak's sequences clustering into nine highly divergent maternal lineages. The current study analyzed the genetic variation and phylogenetic analysis of the IFN-α-A gene in the domestic yak, with comparisons to other IFN regions to investigate immune diversity levels and to design molecular selection strategies for better disease-resistant animals. It found nine clusters, and the implications of these findings can be utilized for yak conservation.

Keywords: Interferon-α-A, phylogeny, microsatellite.

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1. Introduction

Elucidating the origin of domesticated yaks (Poephagus grunnies) will extend our broad understanding of the main ungulate domestications during the history of human civilization. (Wiener et al., 2003), Domestic yaks are mainly distributed in the Qinghai-Tibetan Plateau (QTP), the largest continuous high-elevation ecosystem in the world, occupying nearly 2.5 million km^2 of the Asian continent and reaching an average elevation of more than 4000 m a.s.l. (Buzzard & Berger, 2016).

It has been speculated that wild yaks were first domesticated in Tibet (Fig. 1) because the earliest evidence of human activity and yak husbandry (Qiang Culture), dating from ca. 10,000 yrs BP, has been found in this region. However, because it is difficult to distinguish between wild and domestic yak remains, this assumed domestication time needs to be confirmed by further evidence (Buzzard &

Berger, 2016). The substantial production of meat and milk with a rich protein and fat content compared to other cattle makes them highly significant for humans (Li et al., 2011). Many dairy products are obtained from yak milk, including curds, cheese, butter, ghee, etc. (Olsen, 1990; Jianlin et al., 2000; Wiener et al., 2003; Leslie & Schaller, 2009). The skin, bones, and dung are valuable and used for various purposes (Olsen, 1990; Wiener et al., 2003; Leslie & Schaller, 2009). Domestic yaks are highly important in fulfilling the increasing demand for food as the increasing population demands.

The domestic yak genome also consists of T cell-mediated, an organ-specific autoimmune disease. IFN-α is a member of the type I IFN family and was the first cytokine discovered as well as the first used in human clinical trials and the first approved by regulatory authorities for human use. Receptor-ligand engagement's biological effects are mediating antiviral, antiproliferative, and immunostimulatory responses in cells (Platanias, 2005).

IFN-α is a naturally occurring glycoprotein secreted in response to viral infections by cells and the principal IFN-α-secreting cell in the blood (Pereiro et al., 2008; Mantegazza et al., 2013). Therefore, the present study aimed to construct a phylogenetic tree using the Maximum Likelihood method with the IFN-α-A gene concerning other regions of IFN in Pakistani domestic yak (*Bos grunniens*) to understand the level of immunity act as a critical indicator for conservation.

2. Materials and methods

Domestic yak blood samples were collected from the Gilgit and Bultistan regions. Animals with typical yak phenotype features were also selected from several breeding areas, such as wildlife parks and zoos.

Aseptic blood samples (3 mL) were collected from the jugular veins of domestic yak and kept in 15 mL Falcon tubes containing anticoagulant (200 µL) with ethylenediaminetetraacetic acid (0.5 M EDTA). Blood samples were placed on ice just after collection and brought to the laboratory. Before DNA extraction, samples were stored at -20 °C. All selected and sampled domestic yaks were handled carefully, fulfilling the pertinent guidelines. Animal ID, sex, breed, location of the animal, and age were also recorded.

Genomic DNA extraction from blood samples was performed using the standard protocol, involving the lysis of white blood cells, digestion of protein, and finally, precipitation after isolation of DNA and purification. Dissolved DNA samples in TE buffer (pH 8.0) were stored at -20 °C for use in the future. DNA samples were quantified with the help of agarose gel electrophoresis (2 %); for comparison, a standard DNA/DNA ladder was used. All samples were brought to the same concentration level of 50 ng/µL. To amplify the coding region of Yak IFNa-1, the sequence primers forward: 5' ATG GCC CCA GCC TGG TCC TT – 3' and reverse 5' – TCA GTC CTT TCT CCT GAA ACT CTC C-3' primers were designed using Primer 3 software (Rozan & Skaletsky, 2000) for the IFN-α-A gene from a previously reported sequence available from GenBank, National Centre for Biotechnology Information (NCBI).

For the amplification of the PCR amplification was done on a Thermocycler (Bio-Rad, USA) using a reaction mixture of 25 µL containing 50 ng/µL genomic template, 5U Taq polymerase enzyme (Thermo Scientific USA), 2.5 mM each dNTPs, 2.5 µL ten × buffer and 2.5 mM of MgCl2. PCR condition was used as an initial denaturation of 5 minutes at 95 °C followed by 35 cycles; each cycle consisted of three phases: denaturation for 45 s at 94 °C, following annealing for 45 s at 52 °C and an extension for 45 s at 72 °C were selected in PCR machine. The final extension was carried out at 72 °C for 10 minutes. DNA fragment sizes were determined in an ABI 3130 Genetic Analyzer.

IFN-α-A gene and IFN other genes (401 bp) through a thermocycler (Bio-Rad, USA), genomic DNA, a set of primers, dNTPs, PCR buffer, MgCl2, nuclease-free water, and DNA polymerase were used according to the standard protocol. The PCR product was analyzed through 2 % agarose gel electrophoresis, and the amplified bands were visualized under UV light using a gel documentation system (Bio-Rad). The amplified PCR products were precipitated with 80 % ethanol and dissolved in a final volume of 10–15 µL deionized water. DNA quality was checked on 2 % agarose gel before sequencing using an ABI PRISM 3130 XL genetic analyzer (Applied Biosystems, USA).

The evolutionary history was inferred using the Maximum Likelihood method and the Kimura 2-parameter model (Casanellas et al., 2020). The bootstrap consensus tree inferred from 100 replicates represents the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50 % of bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test 100 replicates) are shown above the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 2.5047)). This analysis involved 53 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1143 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

Different population genetics software packages (Ensemble Variant Effect Predictor (SNP Effect Predictor, Fast SNP, and Predict SNP) were used to check the SNPs, homology, and phylogenetic relationship of domestic yak (*Bos grunniens*) with multiple species.

3. Results and discussion

This study assessed genetic variations in 53 sequences (401 bp) of two yak populations from Pakistan. Through these sequences, all the samples were successfully analyzed. As a matrilineally inherited genetic material, DNA materials have been widely used to study maternal phylogenetic relationships among mammals (Qiu et al., 2012). Genetic diversity is an integral part of all biological diversity. It is the basis of biological evolution and species differentiation and is of great significance for population maintenance and reproduction, and adaptation to habitat changes. Mitotype diversity and nucleotide diversity are critical indicators to measure the degree of genetic variation in the population (Qi et al., 2009).

In this study, the interferon-alpha (IFN-α-A) gene in domestic yak was amplified and sequenced to investigate and identify the genetic variations. IFN-α (IFNA), IFN-β (IFNB), IFN-ε (IFNG), IFN-ω (IFNW), and IFN-τ (IFNT)

six main subtypes found in the analysis, and further, it was clustered into nine sectors. IFN-β (IFNB) for two sub-clusters, IFN-α (IFNA) for two sub-clusters. The result of weak phylogeographical and morphological structuring/correlations within Pakistani yaks is consistent with the previous studies based on DNA analysis (Bailey et al., 2000), microsatellite markers, and blood protein electrophoresis (Tu et al., 1997). Nucleotide and the deduced amino acid sequence of domestic yak IFN-α-A were also compared with IFN-subtypes of the same animals.

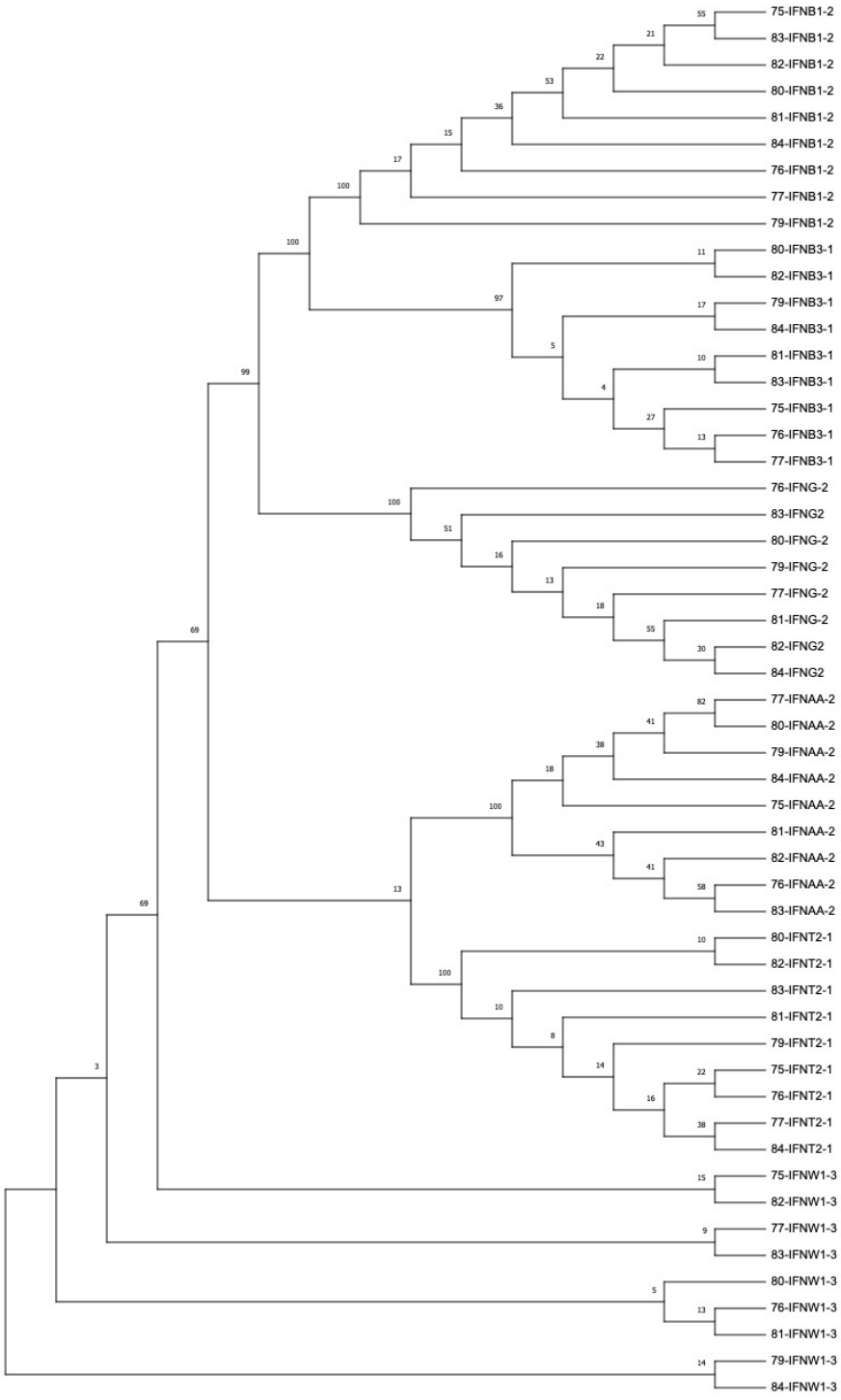

Figure 1. The evolutionary history was inferred by using the Maximum Likelihood method clearly shows nine (9) clusters using a bootstrap value of 100. (IFN-α (IFNA), IFN-β (IFNB), IFN-ε (IFNG), IFN-ω (IFNW), and IFN-τ (IFNT))

The phylogenetic analysis of domestic yak of Pakistan was based on the nucleotide sequence of the IFN-α-A gene and compared with previously published IFN-α-A gene sequences of related species retrieved from GenBank. The evolutionary history was inferred using the Neighbour-joining method (Saitou & Nei, 1987), and evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004). Phylogenetic analysis of the IFN-α-A gene suggested the domestic yak's sequences clustering into nine highly divergent maternal

lineages was found in the Pakistan region (Figure 1). Moreover, the homology of domestic yak with other bovine and caprine species was analyzed through the BLAST (Basic local alignment Search Tool) program. It showed high similarity with Bos grunniens (97.59 %) followed by Bos indicus (97.19 %), Bubalus bubalis (97.02 %), Bos taurus (96.84 %), Bison bison (96.84 %), Capra hircus (95.96 %), Ovis aries (95.26 %), Bos mutus (94.21 %), Antilo pecervicapra (94.04 %), Orcinus orca (87.85 %), Odocoileus virginianus (93.51 %), Sus scrofa (83.22 %) and Homo sapiens (75.85 %) (Table 1).

Table 1
The similarity of the domestic yak IFN-α-A gene with other mammalian species (%)

Species	Common Name	% Identity	Accession Number
Bos grunniens	Domestic yak (China)	97.59 %	JN835446.1
Bos indicus	Zebu cattle	97.19 %	XM_019965693.1
Bubalus bubalis	Water buffalo	97.02 %	XM_025281446.1
Bos taurus	Cattle	96.84 %	XM_002689533.5
Bison bison	American buffalo	96.84 %	XM_010848790.1
Capra hircus	Domestic goat	95.96 %	XM_018052805.1
Ovis aries	Sheep	95.26 %	XM_027963937.1
Bos mutus	Wild yak	94.21 %	XM_005910690.1
Antilo pecervicapra	Blackbuck	94.04 %	FJ959075.1
Odocoileus virginianus	White-tailed deer	93.51 %	XM_020904914.1
Orcinus orca	Killer whale	87.85 %	XM_004275042.2
Sus scrofa	Wild boar	83.22 %	GQ415062.1
Homo sapiens	Human	75.85 %	KP719981.1

As illustrated in our results, the conservation of cattle microsatellite loci in the yak genome indicates the high applicability of bovine microsatellites for genetic diversity, relationship, and parentage analysis in the yak. It can be used for future genetic studies for this critical Asian species.

4. Conclusions

In conclusion, the possible origin, evolutionary history, molecular phylogeny, and selection evidence of domestic yaks (*Bos grunniens*) in Pakistan were explored in this study. The results provided a basis for further enrichment of the background information of these yaks and evaluated their uniqueness.

Conflict of interest

The authors declare that there is no conflict of interest.

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