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Molecular characterization of inhibitor of apoptosis gene (*IAP*-1) in native chicken of Poonch region from international borders of India and Pakistan

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Abstract
Apoptosis of programmed cell death is an important phenomenon of body immune response. Inhibitor of Apoptosis gene (*IAP*-1) is involved in different biological activities like binding and inhibiting caspases, regulating cell cycle progression, and modulating receptor-mediated signal transduction. The present study was undertaken with the main objectives of molecular characterization of *IAP*-1 gene in local native chicken of Poonch region. RNA Purification Kit (HiPura) was use for RNA extraction from Poonchi blood. After cDNA synthesis, *IAP*-1 gene of 544 bp size was amplified from cDNA using specific primers designed by Primer 3 software. Direct sequencing was carried out by Sanger sequencing with both forward and reverse primers to obtain 544 bp sequence product. The sequences were studied by using BioEdit and MEGA X softwares. The sequence results of *IAP*-I gene of Poonchi chicken and Kadaknath chicken were compared with, Leghorn, Fayoumi breed of chicken and also with other sequence of *Gallus gallus* complete Cds, *Centrocercus urophasianus*, Mus *musculus*, *Gallus Gallus partial Cds*, *Rattus norvegicus*, *Tympanuchus phasianellus.* AT content were higher then GC content for *IAP*-1 gene. Sequence results were analyzed for synonymous (S) and non-synonymous (NS) changes. No SNP variation within Poonchi population as well as with Kadaknath population were observed. Fayoumi breed showed 2 (NS) changes. Highest number of NS changes were observed with *Mus musculus*. The Genetic distance result showed no dissimilarity between Poonchi chicken and Kadaknath breed. Highest genetic distance was observed with *Tympanuchus phasianellus.*

Keywords: *IAP*-1, sequencing, ClustalW, genetic distance, Poonchi chicken, India

1. Introduction
The complex immune system of poultry provides an opportunity for investigating polygenic regulation of immune response in chickens (Lamont, 1998) [10]. Genetic resistance to diseases is a great resource for control and prevention of diseases and for improvement of productivity in poultry (Bacon *et al.*, 2000; Bates *et al.*, 1998) [2, 3]. Differences in disease resistance between strains of chickens have long been described (Bearse *et al.*, 1939; Lambert, 1932) [4, 11]. Local native chicken breeds are considered more adaptive and disease resistant than the exotic breeds. In recent years exploration of native germplasm has been a focused area worldwide. However, it is difficult to ascertain whether a single or a set of gene (s) define this property to indigenous chickens. There has been special emphasis on development of improved genetic stock for disease tolerance. Native Indian chicken with better immune competence has been indicated earlier by higher complement activity, higher serum lysozyme level, and antibody response (Haunshi and Sharma, 2002; Baelmans *et al*., 2005) [9, 1]. The inhibitor of apoptosis protein-1 (*IAP*-1) gene is a member of the *IAP* family, which is involved in host ant*IAP*optotic mechanisms (Deveraux and Reed, 1999). The *IAP* prevents apoptosis by binding and inhibiting caspase activity at various steps of the apoptotic signaling pathway (Deveraux *et al.*, 1997; Roy *et al.*, 1997) [6, 13]. Chicken *IAP1* has been mapped to chromosome 1, and its sequence has 85% homology to the human *IAP* gene (Goodenbour *et al*., 2004) [7].
Poonch is one of the remotest districts of the Jammu and Kashmir (UT) and situated on line of Control. This native poultry population is quite hardy and thrives well in adverse climatic conditions. It is very imperative to identify and characterize these native chicken population in order to use them for development of disease resistant and early maturing strains to ameliorate the socio-economic status of economically weaker and the poor resource less nomadic and other farmers of the remote district of U.T of Jammu and Kashmir.

Therefore, the present study was undertaken with the objective to characterize the immune related inhibitor of apoptotic protein (*IAP*-1) in Poonchi chicken.

2. Materials and Methods

Fresh blood sample from Poonchi Chicken were collected from the Brachial Vein in EDTA coated vacutainers. RNA isolation was done using RNA Purification Kit (HiPura). Concentration and purity of RNA was checked using Nano-drop-spectrophotometer. Samples having OD ratio (260nm/280nm) of approx. 2 was used for further analysis. Quality of RNA was checked on 1% agarose gel and visualized under transilluminator. Presence of three intact bands of 28s,18s and 5s with smearing indicated good quality and intactness of RNA. cDNA was synthesized using Revert aid first strand cDNA synthesis kit.

Forward primer 5´- GTAACTACTAGGGCTGCCGA-3´ and reverse primer 5´- AACTCTCCTCCTTTCACACG-3´ were designed using Primer 3 software. PCR was performed in a total volume of 25 µl.

Thermocycler conditions for *IAP-1* gene were 95 °C for 3 min, then 34 cycles of 95° C for 0.30 min, 55 °C for 0.45 min, and 72 °C for 1.30 min, with a final extension step of 5.00 min at 72 °C. Positively amplified PCR products were then checked on 2% agarose gel along with 100bp DNA ladder. The products with size 554 kb were sent to Biologia Research India Pvt Ltd, New Delhi, for sequencing.

2.1 Sequence Analysis

IAP-1 gene sequences for Poonchi chicken were then subjected to BLAST analysis. Sequence was analyzed with MEGAX and BioEdit softwares and compared with other reported sequences of different breeds and species obtained from NCBI (National Center for Biotechnology Information). Multiple sequence alignment was done by ClustalW method using MEGA X software to see the identities, similarities and differences. The phylogenetic tree was constructed using Neighbor-Joining method (Saitou and Nei, 1987) [14], based on the aligned sequences. The evolutionary distances analyses were conducted using the Maximum Composite Likelihood model (Tamura *et al*., 2004) [15]. The relative frequencies of the nucleotide composition were computed for all the sequences used.

3. Results and Discussion

Immune response is mainly controlled by immune system consisting of different cells. It is very important to study and understand the polygenic role of various genes in prevention of disease. Inhibitor of apoptotic protein (*IAP*-1) prevent apoptosis by binding and inhibiting the caspase (Deveraux *et al*., 1997 & 1998; Roy *et al*. 1997; Takahashi *et al*. 1998; Tamm *et al*., 1998) [5-6, 13, 16] which should act in concert to produce an effective response (Pinard-van der Laan *et al*., 1998) [12].

The sequence results of *IAP*-I gene of Poonchi chicken and Kadaknath chicken were compared with, Leghorn, Fayoumi breed of chicken and also with other sequence of *Gallus gallus* complete Cds, *Centrocercus urophasianus*, *Mus musculus*, *Gallus Gallus partial* Cds, *Rattus norvegicus* and *Tympanuchus phasianellus.* Sequencing results showed no SNP variation within Poonchi population as well as with Kadaknath population (Fig. 1a-e). Also, no variation was observed with Leghorn sequence but variation was seen with Fayoumi breed of chicken.

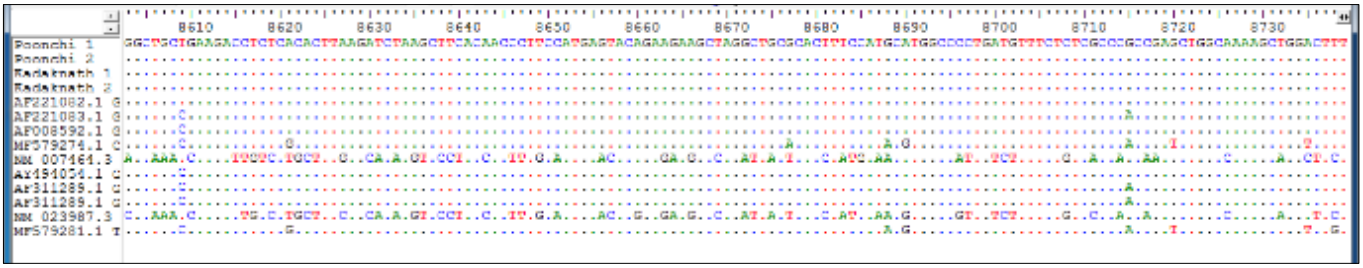

Fig 1a

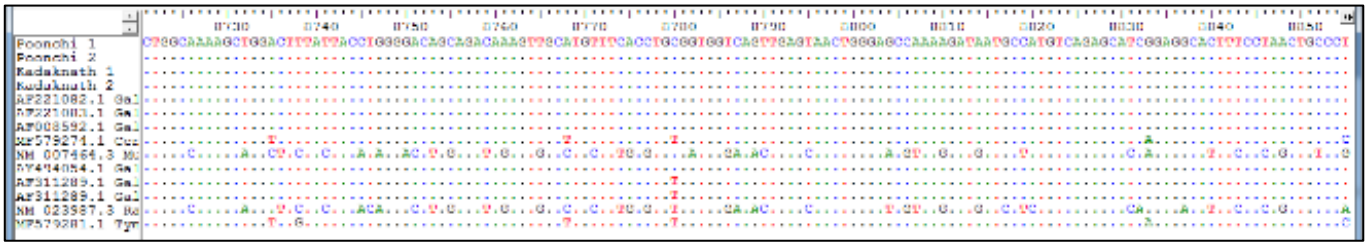

Fig 1b

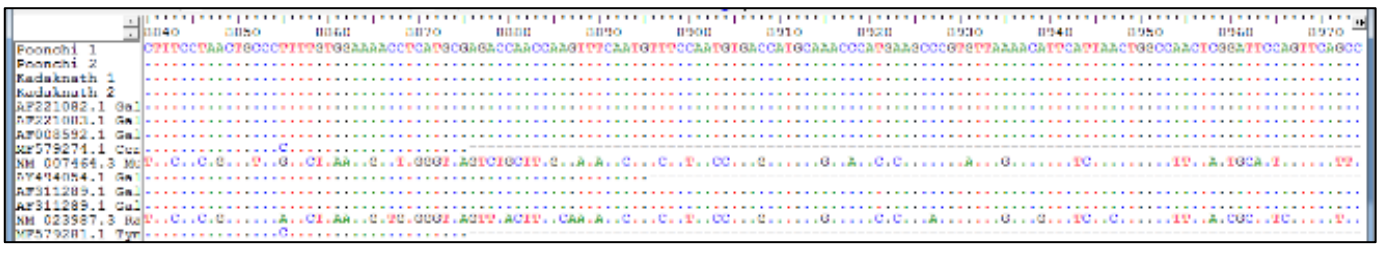

Fig 1c

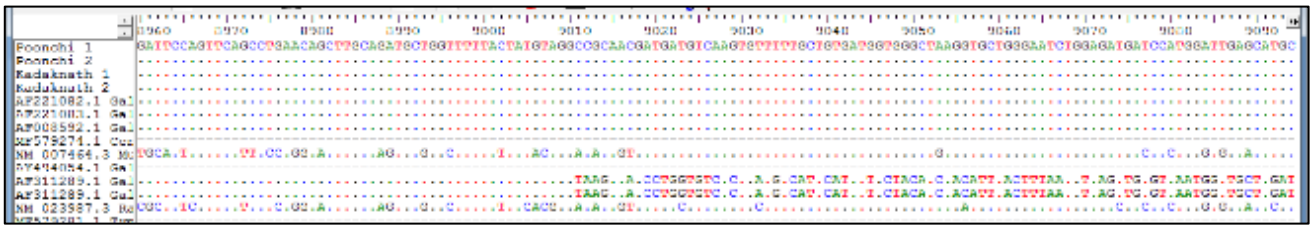

Fig 1d

Fig 1e

Fig 1a, b, c, d, e: shows the nucleotide base pair changes between different breeds and species in the present study

Supakon (2016) studied *IAP*-I gene in Thai native chicken and reported a 394 bp product. PCR-RFLP by using *Bg*II restriction enzymes revealed 3 genotypes and 2 alleles with 0.70 & 0.30; 0.75 & 0.25; 0.72 & 0.28 and 0.77 & 0.23 frequencies in Luenghangkhao (LK), Pradhuhangdum (PD), Chee (CH) and Red Jungle Fowls (RJF), respectively. In contrary to the present finding by sequencing, Supakorn (2016) observed within and between population variations.

You *et al*. (1997) sequenced the full length chicken *IAP*-1 gene and reported SNPs and synonym and non-synonym changes.

Between different species high variations were observed. In the study of nucleic acid composition of Poonchi chicken and other breeds and species it was observed that AT content were higher then GC content for *IAP*-1 gene in all the breeds and species in the present study (Table 1).

Table 1: Nucleotide content for *IAP*-1 gene

	A%	C%	G%	T%	G+C%	A+T%
Poonchi_1	25.45	22.74	25.63	26.17	48.38	51.62
Poonchi_2	25.45	22.74	25.63	26.17	48.38	51.62
Kadaknath_1	25.45	22.74	25.63	26.17	48.38	51.62
Kadaknath_2	25.45	22.74	25.63	26.17	48.38	51.62
Leghorn	25.68	22.96	25.00	26.36	47.96	52.04
Fayoumi	25.89	23.35	24.70	26.06	48.05	51.95
Gallus Gallus complete Cds	28.68	20.16	22.27	28.89	42.43	57.57
Centrocercus urophasianus	24.46	26.93	21.67	26.93	48.61	51.39
Mus musculus	27.84	23.05	24.61	24.50	47.26	52.34
Gallus Gallus partial Cds	25.38	27.41	21.32	25.38	48.73	50.76
Gallus Gallus partial Cds	25.75	22.60	24.13	27.52	46.73	53.27
Rattus norvegicus	28.26	23.03	24.73	23.98	47.26	52.24
Tympanuchus phasianellus	24.46	26.93	21.98	26.63	48.92	51.08

The amino acid sequence results were analyzed for synonymous (S) and non- synonymous (NS) changes. No variations within Poonchi population as well as with Kadaknath population were observed. Synonymous and non-synonymous changes are shown in Fig. 2. Fayoumi breed showed 2 (NS) changes. The highest number of NS changes were seen with *Mus musculus*a (121) followed by with *Tympanuchus phasianellus* (116) changes with Poonchi chicken. Supakorn (2016) reported G/A synonymous SNP in native Thai chicken. Zhou *et al*. (2001) observed T/A substitution using PCR-RFLP.

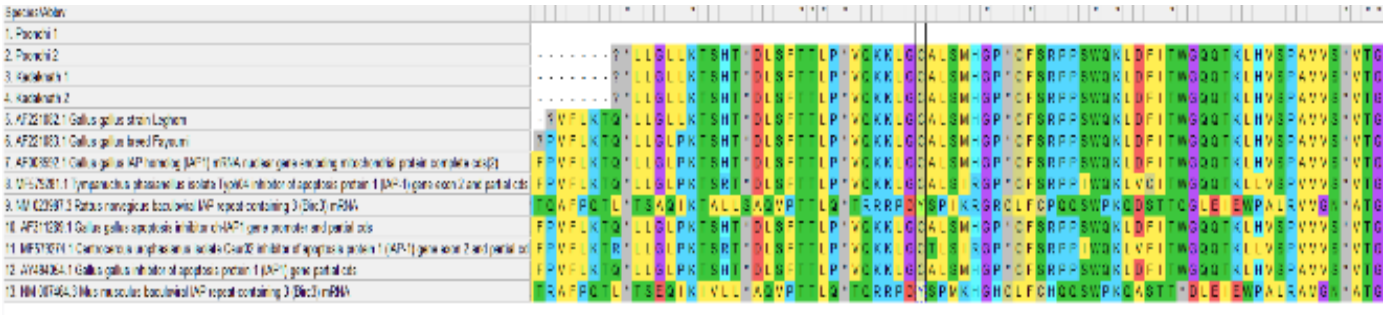

Fig 2a

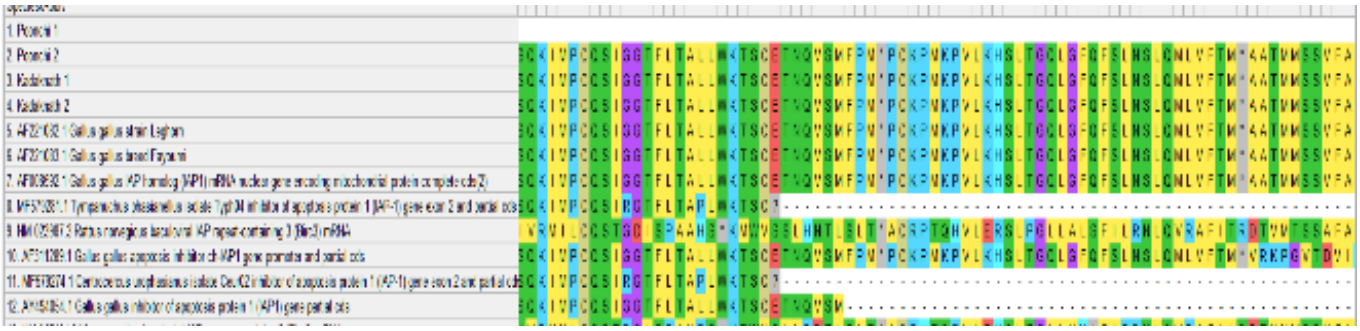

Fig 2b

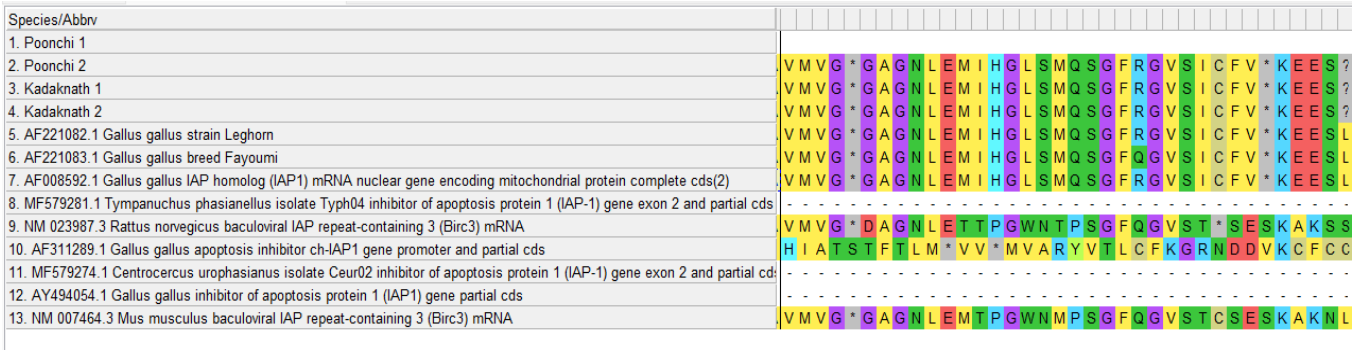

Fig 2c

Fig 2 a, b, c: Shows amino acid changes between different breeds and species in the present study

The genetic distance result showed no dissimilarity between Poonchi chicken and Kadaknath and Leghorn breed. Genetic distance of 0.0097 was seen with Fayoumi breed. The highest genetic distance was observed with *Tympanuchus phasianellus* with value of 1.4851.

BioEdit (Hall, 1999) [8] software was used to construct phylogenic tree and Neighbor Joining method was used to infer evolutionary history. In order to compute evolutionary distance Maximum Composite Likelihood method was used. Deveraux and Reed (1999) constructed phylogenetic tree for amino acid sequence of *IAP*-1 gene.

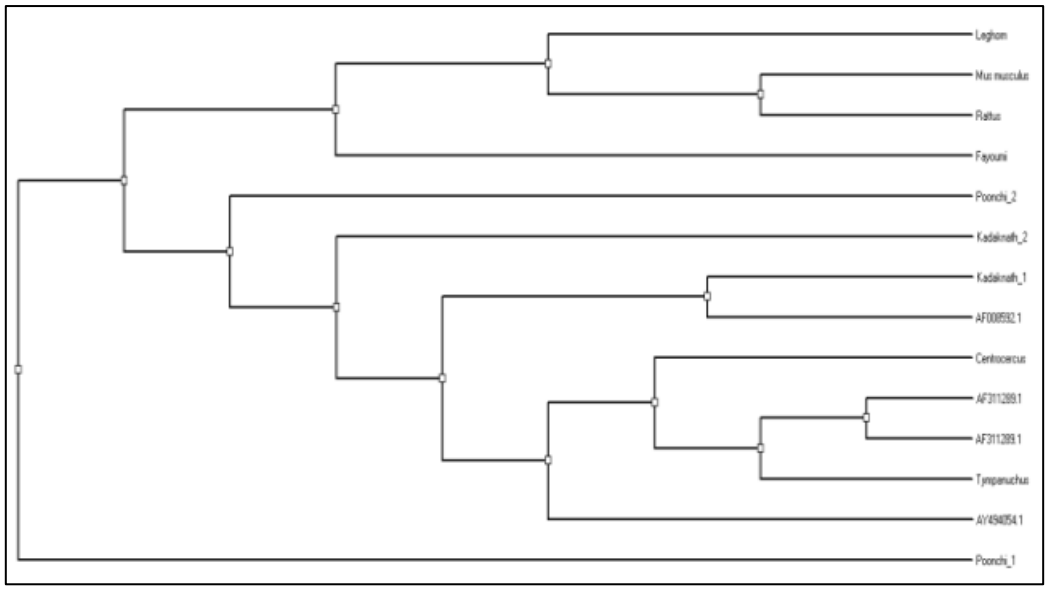

Fig 3: Phylogenetic tree of IAP-1 gene

4. Conclusion

Genetic characterization for *IAP*-1 gene in Poonchi chicken showed no within population variation or any variation with Kadaknath breed but it showed genetic variability between breed and species. Maximum divergence from Poonchi chicken was observed with *Tympanuchus phasianellus*. It can be concluded from the study that there is sustainable variation between the different species and but less variation within the breeds. Genetic characterization on large populations may explore the genetic variability of *IAP*-1 gene in the Poonchi population to establish the disease resistance population for improving the health status of native chicken population.

5. Acknowledgements

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