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Molecular Characterization and Sequencing of Interleukin-15 (*IL15Rα*) in Poonchi Chicken from Jammu and Kashmir

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ABSTRACT

Background: Conservation and promotion of native breeds is important for developing disease resistant and resilient genotypes. Present study is the first study on molecular characterization of immune related genes in Poonchi chicken population of Jammu, India. Interleukin-15 (*IL-15Rα*) is involved in generating immune response by acting as a growth factor in recruiting T and NK cells.

Methods: RNA isolation followed by cDNA synthesis from freshly collected blood samples of Poonchi was done. PCR amplification with specific primers was carried out for *IL-15Rα* gene of 243 bp size. Sanger sequencing was followed by analysis with BioEdit and MEGA X softwares. Variation was observed within the Poonchi chicken population under study and also with other breeds like Kadaknath and Leghorn.

Result: Result showed a single nucleotide polymorphism change of T→C. The amino acid compositions showed that GC content was higher than AT content. The within population genetic distance was 0.0078. Sustainable variation was observed through multiple alignment and genetic distance studies which suggest further association studies should be carried with larger Poonchi chicken population to identify presence and absence of resistant genotypes.

Key words: ClustalW, Genetic distance, *IL-15Rα*, Poonchi chicken, Sequencing, SNP.

INTRODUCTION

Poultry and dairy are considered important allied sectors of agriculture which support agriculture and rural economy (Toor and Goel, 2022). Chicken represents a valuable genetic resource and an important protein source (Kaushik *et al.,* 2023). As compared to exotic breeds, indigenous breeds have always been believed to show better disease resistance, immunocompetence and adaptability (Kundu *et al.,* 1999). This property of indigenous chickens may be due to single or a set of genes. Due to emergence of various bacterial, viral and protozoal diseases, it has become utmost priority to develop breed/strain which are more resistant to adverse climatic conditions in addition to increase productivity. As a proinflammatory cytokine Interleukin-15 (*IL-15*) has a similar biological function to *IL-2.* It also plays many important roles during immune response like inducting and proliferating T cells to tissues, B cells stimulation and immunoglobulin synthesis (Kumaki *et al.,* 1996). Earlier studies have reported the role of IL15 and its receptors in many different disease pathways such as leukemia, bowel disease (Yamada *et al.*, 1998; Bulfone-Paus *et al.,* 1999). Kaiser *et al.* (2002) reported immune system hyperreactivity exhibited by obese strain of chicken suffering from autoimmune thyroiditis due to *IL-15* constitutively up-regulated in these chickens. Poonchi chicken breed is very hardy and well adapted to local climatic conditions. The main aim of the current study was to characterize *IL-15Rα* gene in native Poonchi chicken population.

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MATERIALS AND METHODS

RNA was isolated by Blood RNA Purification Kit (HiPura) from freshly collected venous blood samples of Poonchi chicken. The samples were collected from unrelated birds from different villages of their natural breeding tract of Ponchi chicken. RNA quality was checked on 1% agarose gel. Three intact bands of 28s,18s and 5s was indicative of good quality of RNA. Revert aid first strand, cDNA synthesis kit was used for synthesizing cDNA. The final thermocycler conditions with final volume of 50 µl were 25°C for 5 minutes, followed by 42°C for 60 minutes and at 70°C for 5 minutes. The positive amplified products with size 243 bp were selected for further purification, sequencing and analysis. The amplified PCR products were sequenced by

Sangers dideoxy chain termination method. The samples sequences were six in number. The sequences were grouped into two Poonchi_1 and Poonchi_2. Each group Poonchi_1 and Poonchi_2 was having 3 numbers of sequences. The sequencing was done by both forward and reverse primers to obtain the full-length amplified sequence. The obtained sequence of *IL-15Rα* gene of Poonchi chicken was analyzed by MEGAX and BioEdit software's and compared with other reported sequences of *IL-15Rα* gene in different species. All the sequences were aligned (multiple sequence alignment) using ClustalW method to reveal the nucleotide substitutions. Multiple sequence alignment using MEGA X software was used to see the similarities and difference. Neighbor-Joining method was used to construct the phylogenetic tree based on aligned sequences (Saitou and Nei, 1987).

RESULTS AND DISCUSSION

Poonch is a remote district of Jammu, India located near to line of control and sharing international border with Pakistan. Poonchi chicken is the first native poultry breed from Jammu province being characterized and registered with National bureau of Animal Genetic Resources, India. This study was the first study carried out in Poonchi chicken for characterization of immune related genes. Primer pair was designed using Primer3 software. F- TCAACACCA TCCT GCGTTAC, R- AGATGTCTCTGGT GATGGCA. PCR conditions were standardized for IL-15Rα gene at 95°C for 3 min, then 34 cycles of 94°C for 0.30 min, 56°C for 0.45 min and 72°C for 1.30 min, with a final extension step of 5.00 min at 72°C. Agarose gel (2%) along with 100 bp DNA ladder was used for checking amplified PCR products. Positive PCR product with a size of 243 bp were selcted for further analysis and sequencing. The sequence results of IL-15Rα gene of Poonchi chicken and Kadaknath chicken were compared with other chicken population/breeds and species sequence from NCBI sequences. Nucleic acid composition of Poonchi chicken and other breeds/ species have been presented in Table 1. It was observed that GC content was higher than AT content in Gallus gallus, whereas, in Homo sapienes, *Bos taurus and Ctenopharyngodon idella* AT content was higher.

Sequence analysis of *IL-15Rα* gene

The result showed within and between population variations as shown in Fig 1 to Fig 4. Synonymous (S) and non-synonymous (NS) changes were observed using amino acid sequence results as shown in Fig 5-6. There was within and between population nucleotide changes for *IL-15Rα* gene in Poonchi chicken population. Result showed a single nucleotide polymorphism change of T→C (Fig 7).

Phylogenetic tree analysis

Phylogenetic tree of *IL-15Rα* gene of Poonchi chicken with other sequences was also constructed (Fig 8). The genetic distance comparison study was done to see the within and between population variability. Phylogenic tree was

Table 1: Nucleic acid composition of *IL-15Rα* gene.

Population	A	C	G	T	G+C	A+T
Poonchi_1	26.03	37.60	20.25	16.12	57.85	42.15
Poonchi_2	26.03	38.02	20.25	15.70	58.26	41.74
Kadaknath_1	15.64	20.16	37.86	26.34	58.02	41.98
Kadaknath2	15.64	20.16	37.86	26.34	58.02	41.98
Gallus gallus cDNA	24.72	36.33	20.97	15.36	57.03	40.07
H. sapiens	31.26	17.26	19.13	32.35	36.39	63.16
Bos taurus	34.15	16.77	18.00	31.08	34.76	65.24
Ctenopharyngodonidella	34.30	16.78	17.99	30.94	34.76	65.24
Equus caballus	16.17	27.07	31.20	25.56	58.27	41.73
H. sapiens	31.26	17.26	19.13	32.35	36.39	63.61
Gallus gallus	22.92	32.63	25.72	18.73	58.35	41.65
Gallus gallus	23.15	32.38	26.01	18.46	58.39	41.61

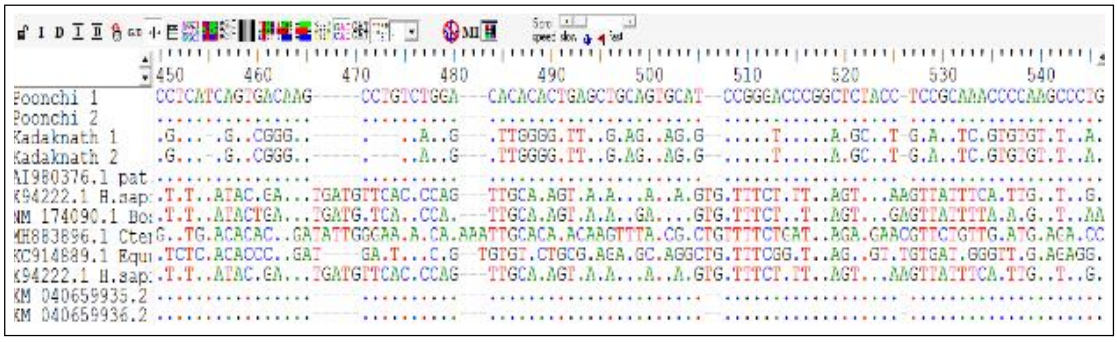


Fig 1: Multiple sequence alignment of *IL-15Rα* gene of Poonchi chicken with other sequences.

constructed using BioEdit software (Hall, 1999) and Neighbor Joining method was used to infer evolutionary history. Maximum Composite Likelihood method was used to compute evolutionary distance. Within population and between breeds genetic distances was calculated (Table 2). The within population genetic distance for poonchi chicken was 0.0078. Poonchi_1 and Poonchi_2 population showed genetic distance of 2.5348 and 2.5712 with indigenous Kadaknath population. *Homo sapiens* were the most distant with a genetic distance value of 3.0053.

The indigenous breeds have the better ability to stand extreme weather conditions, disease resistance and capability to produce even on low input system (Bhave *et al.,* 2020). In the present study *IL-15Rα* gene was characterized

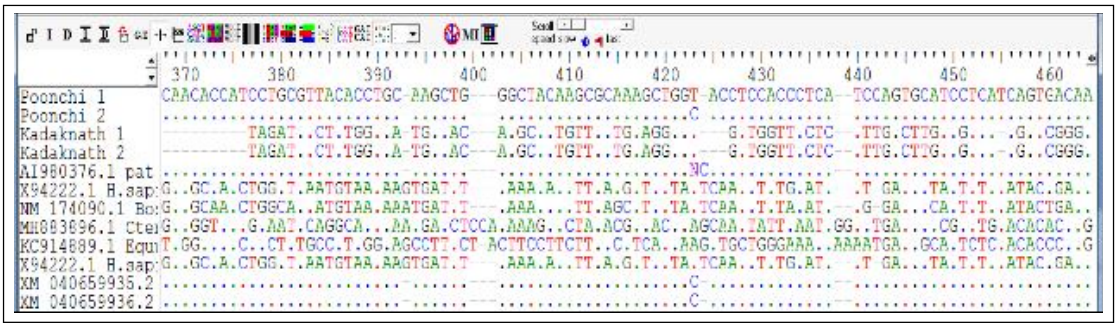


Fig 2: Multiple sequence alignment of *IL-15Rα* gene of Poonchi chicken with other sequences.

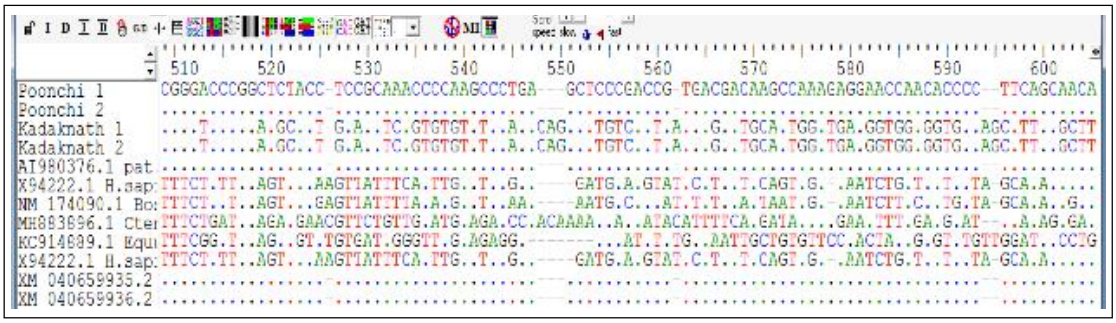


Fig 3: Multiple sequence alignment of *IL-15Rα* gene of Poonchi chicken with other sequences.

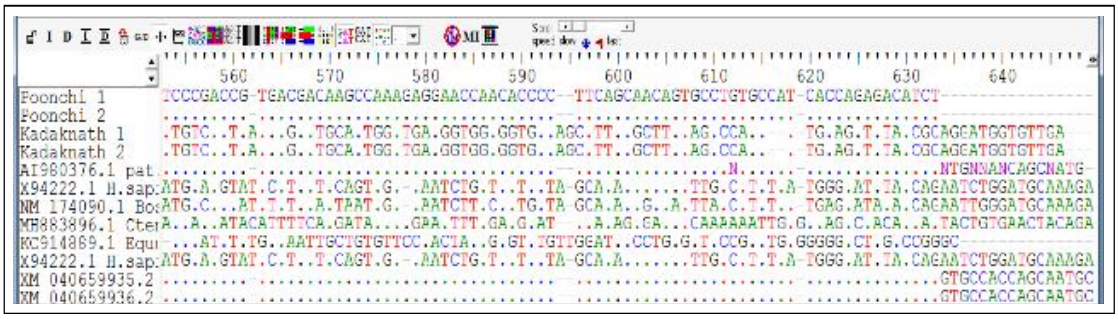


Fig 4: Multiple sequence alignment of *IL-15Rα* gene of Poonchi chicken with other sequences**.**

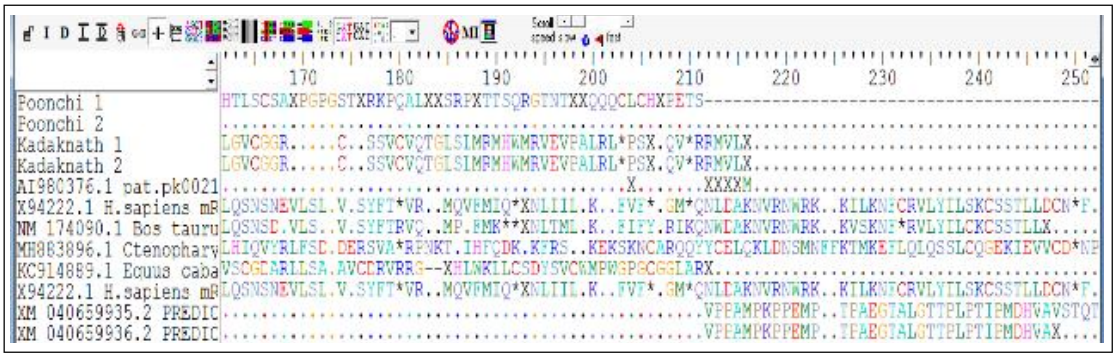


Fig 5: Amino acid changes of *IL-15Rα* gene of Poonchi chicken with other sequences.

in indigenous poonchi chicken population. This was the first study done in poonchi chicken for characterization of immune related genes. There was within and between population nucleotide changes for *IL-15Rα* gene in Poonchi chicken population. Genetic variation is the essential component for genetic improvement. The ClustalW results of immune related gene *IL-15Rα* shows within and between population genetic variability. Sustainable variation was observed through multiple alignment and genetic distance studies which suggest further association studies should be carried on for large population size to identify diseased genotypes. T→C SNP change was detected in the present study although the change was synonymous. Similar polymorphic results were reported by Zhou and Lamount (2003) depicting G→A SNP change between the Leghorn and the Fayoumi lines for *IL-15Rα* gene. Kumar *et al.* (2007) using PCR-RFLP reported similar polymorphism in *IL-15Rα* in Aseel population. In contrast

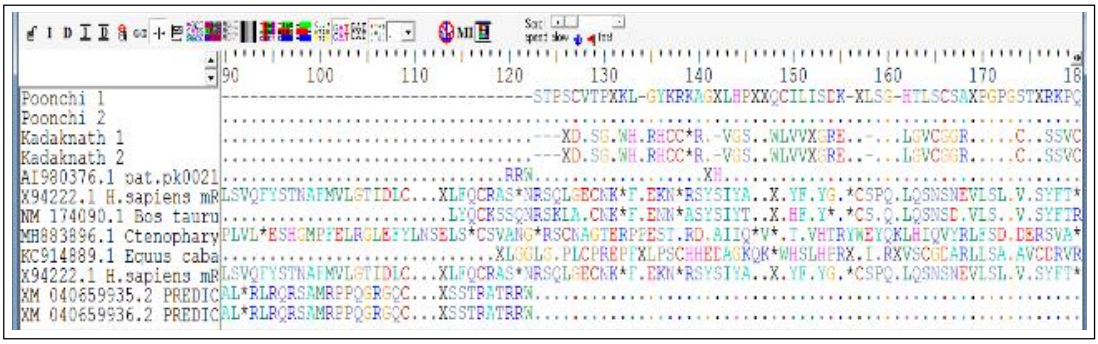


Fig 6: Amino acid changes of *IL-15Rα* gene of Poonchi chicken with other sequences.



Fig 7: SNP changes of *IL-15Rα* gene within the Poonchi.

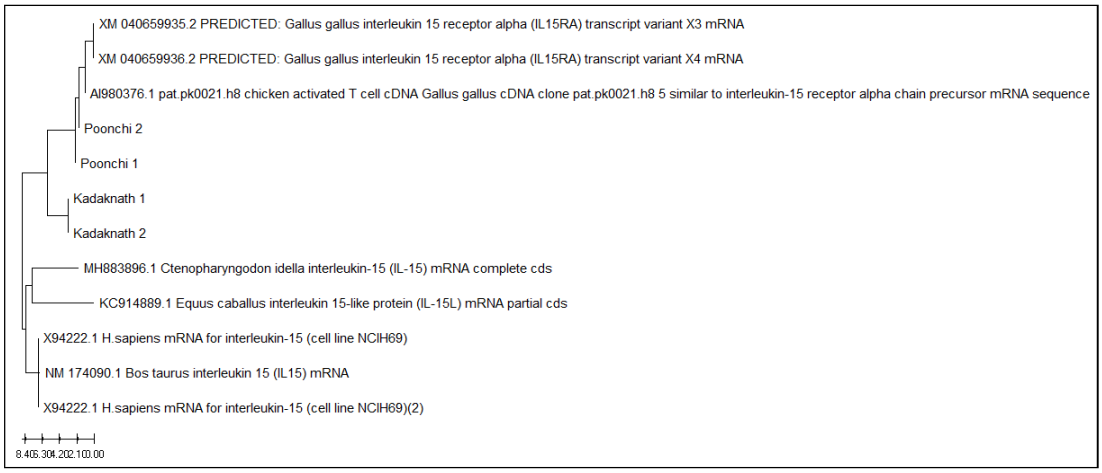


Fig 8: Phylogenetic tree of *IL-15α*.

Table 2: Comparison of genetic distance of *IL-15Rα* gene.

	Poonchi_1	Poonchi_2	Kadaknath_1	Kadaknath2	*Gallus gallusc* DNA	*H. sapiens*	*Bos taurus*	*Ctenopharyng odonidella*	*Equus caballus*	*H.sapiens*	*Gallus gallus*	*Gallus gallus*
Poonchi_1	0.0000	-	-	-	-	-	-	-	-	-	-	-
Poonchi_2	0.0078	0.0000	-	-	-	-	-	-	-	-	-	-
Kadaknath_1	2.5348	2.5712	0.0000	-	-	-	-	-	-	-	-	-
Kadaknath2	2.5348	2.5712	0.0000	0.0000	-	-	-	-	-	-	-	-
Gallus gallus cDNA	2.8459	2.8091	3.1780	3.1780	0.0000	-	-	-	-	-	-	-
H.sapiens	2.9917	3.0053	2.5693	2.5693	3.0320	0.0000	-	-	-	-	-	-
Bos taurus	2.8427	2.8429	2.4565	2.4565	2.5139	2.2983	0.0000	-	-	-	-	-
Ctenopharyng odonidella	2.9219	2.9342	2.5083	2.5083	2.7604	2.0629	2.2672	0.0000	-	-	-	-
Equus caballus	2.4677	2.5031	2.4451	2.4451	2.9566	2.5982	2.7266	2.5279	0.0000	-	-	-
H.sapiens	2.9917	3.0053	2.5693	2.5693	3.0320	0.0000	2.2983	2.0629	2.5982	0.0000	-	-
Gallus gallus	2.5693	2.5693	2.6160	2.6160	3.0796	2.8459	2.8467	2.5267	2.9160	2.8459	0.0000	-
Gallus gallus	2.5693	2.5693	2.6160	2.6160	3.0796	2.8421	2.8467	2.5750	2.9160	2.8421	0.9666	0.0000

to this Singh *et al.* (2008) reported monomorphic *IL-15Rα* in White Leghorn chicken. Jaiswal *et al.* (2009) reported monomorphic nature of *IL-15Rα* gene in Kadaknath native chicken.

The within population genetic distance for Poonchi chicken was 0.0078. Poonchi_1 and Poonchi_2 population showed genetic distance of 2.5348 and 2.5712 with indigenous Kadaknath population. *Homo sapiens* were the most distant with a genetic distance value of 3.0053. Lillehoj *et al.* (2001) compared chicken *IL-2* and *IL-15* for molecular, cellular and functional characteristics and analysis revealed that chicken cytokines had greater homology with mammalian *IL-15* as compared to *IL-2*. The result suggested that chicken IL-15 and IL-2 were potentially capable of enhancing cell mediated immunity. Further studies in large number of samples will be helpful to explore the genetic variation within and between populations to develop the disease resistance or susceptible strains.

CONCLUSION

The immune related (*IL-15Rα*) gene showed variability within and between populations, breeds and species. As this is first study done on Poonchi chicken for immune related gene so further association studies of these genes with some prevalent diseases in large population would be helpful to identify disease resistant/susceptible genotypes in the indigenous chicken population.

Conflict of Interest

There is no conflict of interest on behalf of any author.

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