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RESEARCH ARTICLE
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Identification and sequence analysis of *Tapasin* gene in guinea fowl

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Abstract

Aim: An attempt has been made to identify and study the nucleotide sequence variability in exon 5 - exon 6 regions of guinea fowl *Tapasin* gene.

Materials and Methods: Blood samples were collected from randomly selected birds (12 guinea fowl birds) and *Tapasin* gene amplified using chicken specific primers designed from GenBank submitted sequences. Polymerase chain reaction conditions were standardized so as get only single amplicons. Obtained products were then cloned and sequenced; sequences were then analyzed using suitable software.

Results: Amplicon size of the *Tapasin* gene in guinea fowl was same as reported in chicken with areas of transitions and transversions. The sequence variations reported in these coding sequences might have influence in the protein structure, which may be correlated with the increased immune status of the bird when compared with chicken breeds.

Conclusion: Since *Tapasin* gene is an immunologically important gene, which plays an important role in the immune status of the bird. Sequence variations in the gene can be correlated with the altered immune status of the bird.

Keywords: guinea fowl, Immunity, *Tapasin* gene.

Introduction

Antigen-presenting molecules on antigen-processing cells are specialized receptor glycoproteins coded by an organized cluster of genes that control antigen processing and presentation called major histocompatibility complex (MHC) [1]. MHC, a component of acquired immune system is mediated mainly by T-lymphocytes. All vertebrates, from cartilaginous fish to mammals possess MHC genes and are maintained in the genome as a linked set [2]. During an acquired immune response in the cell, cytosolic antigens are subjected to degradation by a large proteolytic complex, the proteasome, into smaller peptides. To be exposed on the cell surface bound to MHC Class I molecules, the derived peptides are transported inside the endoplasmic reticulum (ER) via the transporter associated with antigen processing (TAP) and loaded onto MHC Class I molecules [3]. This process results from the action of several molecular chaperones (calnexin, calreticulin, ERp57, *Tapasin*) among which *Tapasin* is a specific accessory molecule. This antigen presentation pathway is critically important for immune surveillance against viruses and tumors [4].

Avian MHC is smaller and simpler than that of mammals and is organized differently. It consists of clusters of polymorphic genes located on micro chromosome 16. It is organized in two independent regions designated B and Rfp-Y located on same micro chromosome. Each region contains both Class I and Class II loci. The B region contains three gene clusters whereas the Rfp-Y region contains two. The gene coding for *Tapasin* is present in B region [5].

Tapasin in chicken is present in the MHC (B complex). This gene is located at the centromeric end of the complex, between the Class II *B-LBI* and *B-LBII* genes. Like its human counterpart, it comprises of 8 exons, but features a significantly reduced intron size when compared to the human gene. Chicken *Tapasin* codes for a transmembrane protein with a probable ER retention signal [5,6]. Since *Tapasin* gene plays an important role in deciding the immune status of the bird, a complete genetic study of the gene will throw light on the immune status of the bird. *Tapasin* gene has not yet been characterized in guinea fowl.

Hence, the present study was under taken to identify and compare the sequence variations of the *Tapasin* gene in guinea fowl and chicken.

Materials and Methods

Ethical approval

This study was conducted after approval by research committee and Institutional animal ethics committee

Sample collection and primer design

Blood samples were collected in an anti-coagulant containing microcentrifuge tube from randomly selected birds (12 guinea fowl birds). Genomic DNA was isolated using DNeasy® blood and tissue kit (Qiagen) as per the kit protocol. Primers specific for chicken *Tapasin* gene were designed from the chicken specific *Tapasin* gene sequences available in GenBank (Accession no: AJ004999). Primers (forward (F) 5'

ACGCTGTCCCCGAAGAACCTGGT 3'; reverse (R) 5' CCAACGGATGAGGCCACAGAGGA 3') were designed to amplify the region spanning from exon 5 to partial exon 6 and were custom synthesized (Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore). Amplification conditions were optimized so as to generate single amplicon.

Polymerase chain reaction (PCR), cloning and sequencing

PCR were carried out in a total volume of 25 µl reaction volume with 50 ng of genomic DNA, 10 pmol/µl of each forward and reverse primer, 2.5 µl of 15 mM $MgCl_2$, 2 µl (2 mM d NTPs), and 1 U of Taq polymerase (Bangalore Genei) on a thermal cycler with gradient option. PCR conditions for guinea fowl samples were 95°C for 2 min, 35 cycles of 95°C for 1 min, 68.8°C for 1 min, 72°C for 2 min, and final extension step of 72°C for 5 min. The resulting PCR products were visualized after electrophoresis in 1.5% ethidium bromide-stained 1 × Tris-acetate- ethylenediaminetetraacetic acid agarose gel and the PCR product was extracted from the gel using Gel Extraction Kit (Fermentas, Lithuania).

Gel purified products were then cloned in pTZ57R/T vector system and were sequenced. Sequencing of the recombinant plasmids was carried out using universal primers at the DNA sequencing facility at SciGenome Pvt. Ltd., Cochin Special Economic Zone, Kakkanad, Cochin by the dideoxynucleotide sequencing method using an automated DNA sequencer (Applied Biosystems, USA). Forward and reverse sequences were aligned, and homology between chicken sequences obtained from GenBank and guinea fowl sequences was carried out by BLASTN (http://www.ncbi.nlm.nih.gov/) [7] program of NCBI. Multiple sequence alignment of the sequences obtained were carried out using the Clustal W2 program (http://www.ebi.ac.uk/Tools/clustalW2) [8].

Results

Tapasin: specific primers were designed based on genomic DNA sequences of chicken available in GenBank and all the PCR reactions were carried out using genomic DNA as template. Amplicon size of Guinea fowl *Tapasin* gene was found to be similar to that of chicken i.e. 582 bp (exon 5-325 bp, intron 5-180 bp, exon 6-77 bp) (Figure-1). Comparison with the GenBank submitted chicken sequences (AJ004999) revealed that nucleotide sequences of guinea fowl showed about 97.6% identity. Pair-wise alignment of guinea fowl sequence with AJ004999 sequence showed a sequence variation of 13 nucleotide bases in exon 5, a single nucleotide change in intron 5 and no changes in exon 6. There were 8 transitions at 38, 47, 70, 104, 115, 131, 253 and 383 bp positions (C/T, A/G, T/C, G/A, G/A, A/G, A/G and T/C) and 5 transversions at 45, 62, 66, 77 and 102 positions (A/T, T/A, T/G, C/A and G/C) in the exon 5 and one transition in the intron (A/G) at 383bp (Figure-2). All these replacements might have some co-relation with the protein function in these birds.

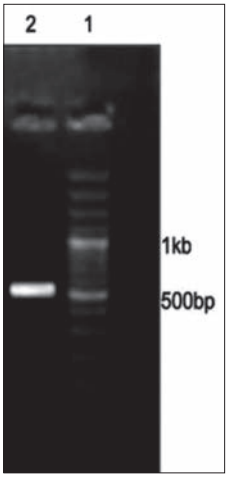

Figure-1: PCR amplified fragment of Tapasin gene in Guinea fowl Lane 1-100bp plus ladder, Lane 2- Guinea fowl *Tapasin* gene (528bp).

Discussion

Poultry production is one of the fastest growing segments of the agricultural sector in India today. While the production of agricultural crops has been rising at a rate of 1.5-2%/annum, the egg and broiler production has been rising at a rate of 8-10%/annum [9]. As a result, India is now the world's third-largest egg producer and the fifth largest broiler producer. The major setback the industry faces today is the onset of various infectious diseases and the control measures to be adopted. Although the immune system of birds differs in several important aspects from that of mammals, the presence of MHC is a unifying feature. The MHC encodes several immunologically relevant proteins, among them classical MHC Class I molecules that are membrane-anchored proteins involved in the presentation of foreign or self-protein-derived peptide antigens [10]. The classical MHC Class I molecules are found on almost all cells, and are recognized by CD8 bearing T cells, most usually cytotoxic T-lymphocytes (CTL). CTL kill transformed cells and viral infected cells, preventing replication of tumor cells or release of the pathogen [11]. The intracellular loading of newly synthesized Class I MHC molecules with peptides in the ER requires the co-operative function of several co-factors, including the TAP, calnexin, calreticulin, Erp57, and the dedicated chaperone *Tapasin* [12].

Keeping in mind the importance of *Tapasin* in the loading of MHC Class I molecules with peptide and the importance of polymorphic variation in antigen processing and presentation machinery, the present study of characterization of exon 5 - exon 6 region of *Tapasin* gene was designed to unravel the homology and variations in the nucleotide sequence of the gene between different poultry species. The protein

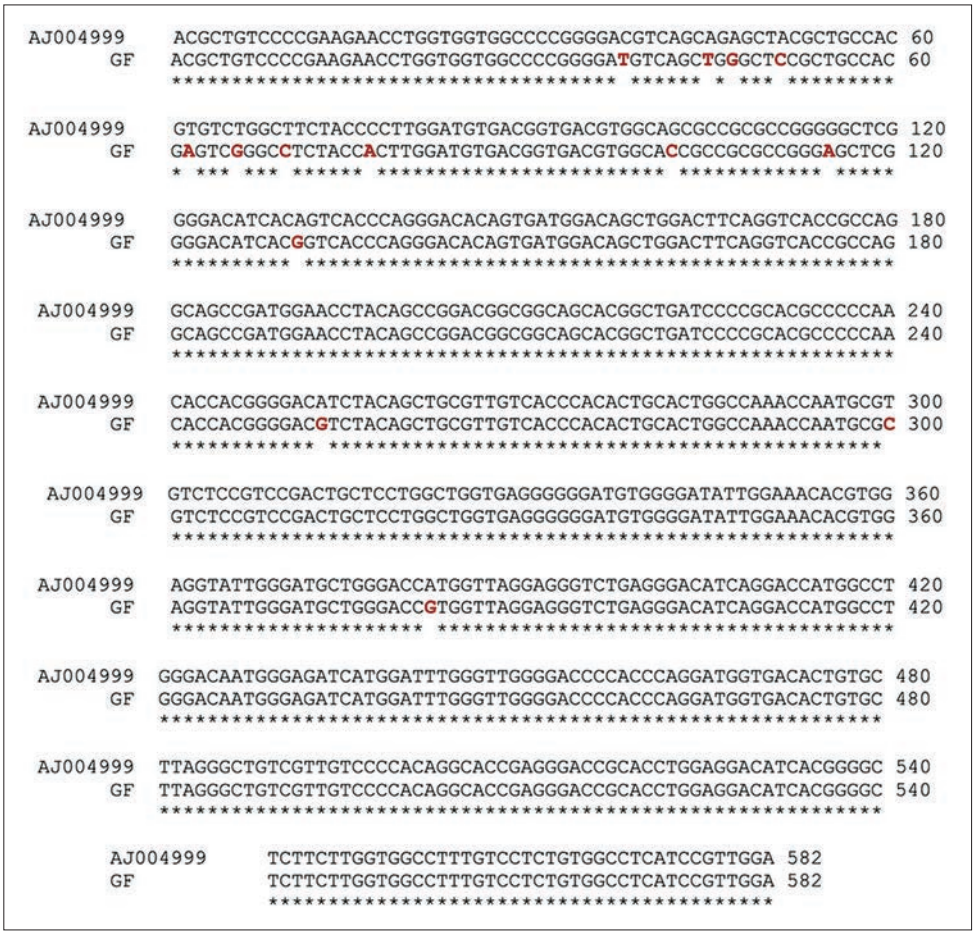

Figure-2: Multiple sequence alignment of AJ00499-Genbank sequence with GF- Guinea fowl sequence.

Tapasin having unambiguous role in immunity to viral diseases and tumors, a complete study of this gene and its protein structure is a pre-requisite to further long range studies.

Guinea fowl comes under family numidae under order galliforms. It is already reported that these birds are resistant to the common diseases occurring in chicken. Guinea fowl is gaining popularity as ideal bird for rural poultry production in India [13]. In this present scenario, a molecular biology approach is needed to investigate immunologically important genes. Majority of variations in nucleotide sequences of guinea fowl were seen in the exon 5 region which is one of the Ig superfamily related domains of *Tapasin* [14-16]. Nucleotide sequence variations in these coding regions may bring about amino acid sequence change, which may influence protein structure and function [17-19]. All these sequence variations might be responsible for the sturdy immune status of the guinea fowl, which is refractive to common poultry diseases when compared to chicken and poultry species.

Conclusion

The present study could help in identifying partial *Tapasin* gene in guinea fowl. Sequence analysis of the gene revealed that the sequence variations noticed in this highly polymorphic region could have played an important role in disease resistance when compared to other poultry species.

Author's Contributions

UR designed and planned the study. VPP carried out the study under the guidance of UR. Both authors participated in draft and revision of the manuscript. Both authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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