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Genetic Polymorphism of TAP1, TAP2 and Tapasin Genes in Exotic and Indian Native Chicken Breeds

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ABSTRACT

Background: Indigenous chicken breeds have shown resilience to common poultry diseases due to their genetic background. Research has highlighted the role of certain genes in the immune response and disease resistance in poultry. Among these, Tapasin and Transporter associated with antigen processing (TAP) genes have been identified as crucial players in the immune surveillance of avian species. However, very diminutive information is available on the genetic polymorphism of TAP1, TAP2 and Tapasin genes in Indian native chicken breeds

Methods: A total of 362 birds from three distinct breeds, namely Brown Nicobari (female: 55, male:64), Ghagus (female: 58; male:60)and Dahlem Red (female: 57; male:68), were used in the study. DNA isolation was performed, followed by PCR amplification using various primers from the three genes. Single-Strand Confirmation Polymorphism (SSCP) analysis was carried out on the PCR products to identify any potential single nucleotide polymorphisms (SNPs). These SNPs were then confirmed through sequencing.

Result: The study has confirmed that these genes are polymorphic in all the three studied chicken breeds. In the exon 7 of TAP1 gene, a polymorphic SNP at nucleotide position 3698(GAG 336 AAG) was identified. A non-synonymous substitution at nucleotide position 4209 (GCA 371 ACA) in the exon8 of TAP1 gene was observed. In exon 6 of TAP2 gene, three variations were observed and of which the variation at nucleotide position 2658 (GAC 446 AAC) is non-synonymous. In exon 7 and 8 of TAP2 gene, a total of three SNPs were observed of which a non-synonymous substitution was observed at nucleotide position 3125(AGG 579AAG) in exon 7. In exon 5 of Tapasin gene, three non-synonymous substitution at nucleotide 3226 (ACG 293ATG), 3292 (CAC 315 CGC) and 3466 (ATT 373ACT) were identified.

Key words: Brown Nicobari, Dahlem Red, Ghagus, Tapasin, TAP1 and TAP2.

INTRODUCTION

The Indian native chicken are considered to have enhanced immunocompetence traits compared to exotic chicken breeds (Yadav *et al.*, 2018). Various genetic factors including SSR markers have been found to affect the immune response/disease resistance (Yadav *et al.*, 2022; Sivalingam *et al.*, 2024) and the growth performance in chicken (Yadav *et al.*, 2019). The genetic variation in the Mx gene has been reported to be associated with the antiviral activity (Ramasamy *et al.*, 2018). Like other vertebrates, chicken also possess Major Histocompatibility Complex (MHC) genes that are maintained in the animals genome as a linked set along with their associated molecules plays a major role in the process of antigen presentation to T cells. For proper functioning of the newly synthesized Class I MHC molecules it has to be loaded intracellular in the endoplasmic reticulum which require the help of many co-factors like Tapasin, TAP, Erp57 and calnexin (Bauer *et al.*, 2011). The available literature suggests that Tapasin and TAP genes are immunologically important genes and are responsible for MHC class I binding thereby plays a vital role in determining the immune status of the birds. A comprehensive study about the genetic variation of these genes will throw light on the immune status of the birds. Tapasin gene is present in the B region of centromeric end of the complex, between the Class II B-LBI and B-LBII genes (Jacob, 2000). Chicken Tapasin gene is localized in the MHC between the 2 class IIβ genes (Sironi *et al.*, 2006). It codes for a trans-membrane protein which has endoplasmic retention signal (Kaufman, 2008) and in mammal it is a 48-kDa transmembrane glycoprotein localized inside the endoplasmic reticulum. Tapasin is essential for the assembly and stabilization of major histocompatibility complex class I molecules (Grandea and Van Kaer, 2001; Brocke *et al.*, 2002) and also acts as a bridge between MHC I and TAP (Sadasivan *et al.*, 1996).

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The chicken TAP and Tapasin genes are located very close to the MHC class I genes, that are found to be highly polymorphic (Kaufman, 2013). The two transporters associated with antigen processing (TAP1 and TAP2) genes in chicken are located head to head between two classical class I genes and contain 11 and 9 exons, respectively (Kaufman *et al.*, 1999). It was reported that exons 6 and 7 of TAP1 and exons 4, 5 and 6 of TAP2 genes have the high rate of variations in poultry (Sironi *et al.*, 2008). Higher degree of polymorphism in TAP1 and TAP2 gene sequence in chicken was also reported by Walker et.al (2011). In the MHC-I pathway, the expression of TAP1, TAP2 and Tapasin (TAPBP) genes were found to be significantly increased when compared to chickens not infected with Marek's disease indicating that the host antivirus responses were generated to enhance antigen presentation (Lian *et al.*, 2010). Very scanty information is available on the polymorphic status of Tapasin and TAP genes in Ghagus, Nicobari and Dahlem Red chicken breeds. The present study was undertaken to study the genetic polymorphism of TAP1, TAP2 and Tapasin genes in the Indian native and exotic chicken breed.

MATERIALS AND METHODS

Experimental birds rearing and management

In this present study, a total of 362 birds from 3 breeds, native [Brown Nicobari (N=119), Ghagus (N=118)] and Dahlem Red (N=125), maintained at ICAR-Directorate of Poultry Research, Hyderabad were used. The experiment was conducted as per the approved guidelines of the Institute Animal Ethics committee (IAEC/DPR/16/1).

Sample collection

Blood samples (n = 362) from all the three breeds were collected from wing vein taking aseptic precautions from the birds of 11 weeks of age. The standard phenol-chloroform method of DNA extraction was followed (Sambrook and Russell, 2006). The primers for the different exons of studied genes were designed using sequence available in the public domain (Table 1).

PCR amplification, SSCP and genetic polymorphism

PCR conditions were optimised for different exons of the Tapasin, TAP1 and TAP2 genes. SSCP analysis was carried out for different PCR amplicons for identification of the variants. The amplicons of the unique SSCP petterns were custom sequenced. The nucleotide/deduced amino acid sequences were analyzed using BLASTn/ BLATp (Altschul *et al.*, 1990; Altschul *et al.*, 1997), Clustal W(Thompson *et al.*, 1994), Sequence manipulation suite (http://www.bioinformatics.org/sms/) and other NCBI data base tools (https://www.ncbi.nlm.nih.gov/). The sequence alignment was carried out using the same reference sequences that has been used for primer designing (Table 1).

RESULTS AND DISCUSSION

The TAP and Tapasin genes of chicken are located near to the class I genes with high levels of genetic polymorphism (Kaufman, 2013). These genes plays a pivotal role in MHC class I-restricted antigen presentation.

TAP1

In the TAP1 gene, exons 1, 2 and 3 were monomorphic in the all the three pedigreed population *i.e.* Brown Nicobari, Ghagus and Dahlem Red. Sun *et al.*, 2012 reported A/G polymorphism in exon 3 of the TAP1 gene in 17 day old pig that showed significant association with the absolute lymphocyte count which are an important components of the host immune response. In exon 4 of TAP1 gene two mutations T to G (at nucleotide position 1822) and T to C (GGT 130 GGC, at nucleotide position 1828) were observed and both of which were synonymous. Exon 5 and 6 of TAP1 gene were monomorphic in all the populations studied. Exon 7 of TAP1 gene was polymorphic in the all the three chicken population (GAG 336 AAG at nucleotide position 3698). The chicken TAP1 and TAP2 genes are highly polymorphic as reported by Sironi *et al.*, 2006, a total of eighteen putative SNPs were identified in exon 6 and 7 of TAP1 gene. Exon 8 of TAP1 gene was polymorphic in all the three population (GCA 371 ACA (Nucleotide position 4209) resulting in non-synonymous substitution (Alanine 371 Threonine). The Exon 10 of TAP1 gene was monomorphic in all the three breed studied. Walker *et al.*, 2011 reported very high level of allelic polymorphism in TAP1 gene in chicken. They reported 41 silent and 14 replacement changes in TAP1 gene. It was reported that chicken Tapasin and TAP1 and TAP2 gene are highly polymorphic with optimum sequence diversity while in human in its monomorphic (Kaufman, 2018). It was reported that the TAP polymorphism does not have any influence on the peptide selection or disease progression and autoimmunity (Obst *et al.*, 1995).

TAP2

Alignment of nucleotide sequences of the exon 1, 2, 3 and 9 of TAP2 genes revealed the highly conserved coding region in all the three chicken breed. In exon 6 of TAP2 gene, three variants were detected at 2658 (GAC to AAC), 2693 (TAT 457 TAC) and 2711 (CGG 463 CGA). The deduced amino acid sequences of these three variants of exon 6 in the TAP2 gene revealed that nucleotide substitution at position 2658 (Codon position GAC 446 AAC) was non-synonymous leading to change of the amino acid from Aspartic acid (Asp) to Asparagine (Asn). The other two substitutions were observed to be synonymous. Sironi *et al.*, 2006 has reported a total of twenty-four putative SNPs in the exon 4 to 6 region of TAP2 gene of Chicken. In the exon 7 of TAP2 gene two variants were identified at 3125 (AGG 579 AAG) and 3132 (GCG 581 GCA) of which the non-synonymous substitution was observed at nucleotide

Table 1: List of primers for TAP1, TAP2 and Tapasin.

Exon	Sequence 5′- 3′	Amplicon size (bp)
	TAP1 (ENSGALG00000035075)	
Forward_Exon_1	TGCATCGCCTCAGCCAATG	200
Reverse_ Exon 1	CGTACCCAGCGCTGAAGC	
Forward_ Exon _2 and 3	GCGAGATGGCCGTGCCCTA	320
Reverse_ Exon _2 and 3	CGGTCCCCCTCACCGGC	
Forward_ Exon _4	GGGATGTGGCCATGCGG	211
Reverse_ Exon _4	CATACCTGCCGGAAGTGC	
Forward_ Exon _5	GCCCTGGCACCACAGATGC	208
Reverse_ Exon _5	CATCCCATACACCACTGGTC	
Forward_ Exon _6	TGGTGACCTCATGGCATC	163
Reverse_ Exon _6	CTCAGGCTCACCCTCAG	
Forward_ Exon _7	AGGTCCTGCTCGACTACTTC	189
Reverse_ Exon _7	CCTTGAGGACGGGTTCCTG	
Forward_ Exon _8	GGCGTATCACTGGAGCTG	175
Reverse_ Exon _8	CCTGGCGGCACAGGTAG	
Forward_ Exon _10	CGCAGAGGTGGGCGAGTTG	152
Reverse_ Exon _10	GACATCCCACCTGCTGCTG	
	TAP2 (AB426152)	
Forward_ Exon _1	GCATTGCTCACCTGGAGC	269
Reverse_ Exon _1	CTGCTGGCAGAGGCCAG	
Forward_ Exon _2and3	CTCGCTGTTTGCCGGCT	407
Reverse_ Exon _2and3	CTGGTGCCGGGTGTCATAG	
Forward_ Exon _6	GCACTGGCGTACTCCTATG	363
Reverse_ Exon _6	CTGGCGGTGCAGGTAGCG	
Forward_ Exon _7-8	GTGGCACTGGTGGGGCAG	338
Reverse_ Exon _7-8	CACTGGTGGCTTCGTCGAG	
Forward_ Exon _9	TCGACGAAGCCACCAGTG	299
Reverse_ Exon _9	TCAGTGCTGTAGCAGCCG	
	Tapasin (ENSGALG00000008022)	
Forward_ Exon _3	GACCCCTTCATCCCTCTCAC	296
Reverse_ Exon _3	ACCCCAAGTTCCCCCTTAC	
Forward_ Exon _4	ACCTCCCCATGTCTTCACAG	377
Reverse_ Exon _4	ACATGGACGCACCAAAGAC	
Forward_ Exon _5	GGCTATATGGGACCGATGTC	405
Reverse_ Exon _5	TTTCCAATATCCCCACATCC	
Forward_ Exon _6 and 7 and 8	TGCTTAGGGCTGTCGTTGTC	439
Reverse_ Exon _6 and 7 and 8	AGCTGGAGTGGAGGTCACTG	

position 3125 (codon position AGG 579AAG) leading to change in amino acid Arginine to Lysine. A substitution effect was identified at nucleotide 3301 (TCG/TTG) in exon 8 of TAP2 gene which was observed to be synonymous. The TAP2 gene was reported to be highly polymorphic and a total of 28 silent and 24 replacement changes were reported (Walker *et al.*, 2011).

Tapasin

In the exon 3 of Tapasin gene, at 1586 bp, a variant was identified that was found to be synonymous, whereas the Exon 4, 6,7 and 8 were found to be highly conserved between and across the breed. The exon 4 of chicken Tapasin gene is highly conserved across the breed while it was reported that a non-synonymous SNP (rs2071888) in exon 4 of the human Tapasin gene causing a change in arginine to threonine (Olloquequi *et al.*, 2022). Copeman *et al.*, 1998 reported a polymorphism in the Tapasin gene, with two alleles encoding arginine or threonine at peptide position 240 in mutant cell line, 220 while in the present study exon 4 is conserved within and across the studied breeds. Bukulmez *et al.*, 2005 reported *TPSN* gene polymorphism in exon 4 that results in a non-conservative amino acid substitution of Arg/Thr at amino acid 260 which has association for susceptibility to Juvenile rheumatoid arthritis. In the exon 5, three variants at nucleotide positions 3226, 3292, 3466 were observed all of which were found to be non-synonymous and the respective amino acid

changes at codon position 293 (ACG to ATG -Threonine to Methionine); 315 (CAC to CGC -Histidine to Arginine) and 373 (ATT to ACT - Isoleucine to Threonine). Sironi *et al.*, 2006 reported that exons 5 and 6 of the Tapasin gene were highly polymorphic in chicken, Turkey and Pheasant which supports the present study. A nucleotide substitution in the intron 7 at nucleotide position 4052 was observed and found to be synonymous. In the other species also, Tapasin gene has been reported to be polymorphic. Williams *et al.*, 2000 reported four new mutations in the intron 4 of human Tapasin. In sheep, a total of twenty-six SNPs were reported in Tapasin gene (Subramaniam *et al.*, 2010).

CONCLUSION

The genetic variations of peptide loading complex gene's influence on the immunocompetence traits in chicken will help in poultry selection and breeding programs having desired disease resistance traits. The present study showed the existence of genetic polymorphism of TAP1, TAP2 and Tapasin genes in the studied chicken breeds. The genetic polymorphism of Tapasin, TAP1 and TAP2 genes identified in the study may further be exploited in the selection and breeding programs of Indian chicken breeds.

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Conflict of interest

The authors declare no conflict of interest.

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