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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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Nucleotide Sequence Variation in MHC Class I Region in Guinea Fowl

S.K. Singh[1], S. Mehra[1], S.K. Shukla[1], Vinay Kumar[1], A. Tiwari[1], M. Mehra[1],
Giriraj Goyal[1], Jose Mathew[2] and Deepak Sharma[1]
[1]Genome Mapping Laboratory, Central Avian Research Institute, Izatnagar 243 122, India
[2]Department of Biotechnology, Bundelkhand University, Jhansi, India

Abstract: The MHC class I gene was amplified, cloned and sequenced in guinea fowl using the primers specific to BF2 gene in chicken. The nucleotide sequence of 571 bp partial CDS of BF2 gene includes 32 nucleotides of signal peptide (exon 1), complete α_1 domain (270 nucleotides) and 269 nucleotides of α_2 domain. For α_1 and α domain no sequence variation was observed within guinea fowl sequences, however, high variability was observed within the other poultry species (15.93-28.03%) except chicken (7.95-9.16%). Between the guinea fowl and other poultry species, the α_1 domain showed high nucleotide variability (29.26-43.70%). Among poultry species, guinea fowl showed least variability with chicken and maximum with duck. Among the substitutions, majorities were of non-synonymous (76.27%) with a ration of 1:3 between synonymous to non-synonymous substitutions. Guinea fowl showed lower genetic distances (Kimura 2-parameter) with chicken and quail (0.211-0.215), while with duck and goose, it showed higher genetic distances (0.343-0.350). Phylogenetic tree, based these genetic distances revealed two major clusters, comprising of guinea fowl, quail and chicken in one with guinea fowl as separate branch, while duck and goose in other.

Key words: MHC I, guinea fowl, phylogeny, nucleotide variability

INTRODUCTION

Major Histocompatibility Complex (MHC) class I molecules have highly polymorphic class I heavy chain (α chain) and a non-covalently associated non-polymorphic light chain (β_2-microglobuin or β_{2m}) and are expressed on the surface of virtually all cells of the body. In chicken genes encoding class I molecules are present in MHC proper or B locus and in non-MHC region, known as *Rfp* Y or Y locus. In B locus, two class I molecules genes i.e. BF1 and BF2 are present, among which the BF2 is predominantly expressed in chicken. The BF2 gene has been well characterized in chicken. The BF2 gene has been characterized in different B haplotypes in White Leghorn, in commercial broilers (Livant *et al*., 2004) and in native chicken lines (Yan *et al*., 2005; Lima-Rosa *et al*., 2004). Among other economically important poultry species, MHC class I genes are very well studied in quail, where multiple class I loci i.e. *Coja* -A, -B, -C and -D were reported (Shiina *et al*., 1999). Xia *et al*. (2004) cloned the MHC class I gene in duck and classified the Anpl-MHC I family genes into four lineages (Anpl-UAA, -0UBA, -UCA and -UDA). Later, Xia *et al*. (2005) also characterized MHC class I genes in goose (Ancy-MHC I). Based on genetic distance, they grouped the Ancy MHC I genes from six individual into four lineges (Ancy-NA, -NB, -NC and -ND). In all these species, the MHC class I genes retained characteristic features of functional MHC class I antigen presentation molecules along with high polymorphism in the amino acid residues in the peptide binding reasons.

Guinea fowl (*Numida meleagris*), a galliformes bird under the family Numididae differ considerable from chicken not only in behaviour, but also in productive and disease resistance abilities. Guinea fowl is an integral part of the agriculture system in African countries (Muchadeyi *et al*., 2004) and is also gaining popularity as ideal bird for rural poultry production system in several Asian countries including India. The reports on MHC class I genes are lacking in this poultry species. Hence the primary aim of our work was to study sequence homology of MHC class I gene between guinea fowl and other poultry species and to use this variability for establishing phyolgenetic lineage of MHC class I gene in these species.

MATERIALS AND METHODS

Guinea fowl: Randomly selected healthy guinea fowl birds (12 week old) from a closed flock population of Lavender variety were used. This population is being maintained as a pedigreed population under half sib family structure over 15 generations and the inbreeding is being kept at minimum by avoiding close relative mating.

Amplification, cloning and sequencing: The cells used for total RNA isolation were monocytes. The monocytes were separated from the blood using LSM[3] and cultured

Corresponding Author: Deepak Sharma, Genome Mapping Laboratory, Central Avian Research Institute, Izatnagar 243 122, India

in RPMI-1640 Medium[4] supplemented with Fetal Bovine Serum and the cells were stimulated with CON A (mitogen) for 1 h at 37°C in CO_2 incubator (5%). The cells were then harvested and the total RNA was isolated using RNAgents™ - Total RNA isolation system[5] and was reverse transcribed using the RevertAid™-first strand cDNA synthesis kit[6].

The forward primer (5'- GCT GCT CGC CGC CGT GTG-3'), derived from 5' end of exon 1 and reverse primer (5'-CCC AGC TCA GCC TTC CCG TA-3') from 3' end of exon 3 were synthesized. The PCR was performed in a total volume of 25 µl containing 2 µl cDNA, 1.5 mm $MgCl_2$, 50 mm KCl, 10 mm Tris-HCl, pH 8.8, 0.1% Triton X-100, 0.01% gelatin, 200 µm of each dNTP, 1 unit of Taq DNA polymerase enzyme (Promega) and 10 Pico mole of each forward and reverse primer. Amplification conditions were: 94°C for 3 min and 35 cycles of 45 s at 94°C, 45s at 56°C and 1 min at 72°C and final extension of 10 min at 72°C. The PCR products were analyzed on 1.6% agarose gel followed by ethidium bromide staining and visualized under ultraviolet light. The PCR products were purified from gel using QIAquick Gel Extraction Kit[7]. The purified PCR products were cloned into the pTZ57R/T vector[6]. The positive clones were identified initially by colony PCR and subsequently by insert release after *EcoR* I and *Pst* I double-digestion of plasmid DNA. Two representative clones from 4 birds were sequenced on both strands by M13 forward and reverse primers on automated ABI PRISM 3100 advant genetic analyzer (Applied Biosystem, Foster City, CA, USA).

Sequence analysis: The sequences obtained were first checked manually and blasted (www.ncbi.nlm.nih.gov/BLAST) to ascertain that sequences were of BF2 gene. The related sequences identified from blast results were retrieved from Genbank (www.ncbi.nlm.nih.gov). These sequences were edited and the concerned region i.e. α_1 and α_2 domains were cut and saved. Subsequently, the sequences from were α_1 and α_2 domains were aligned using CLUSTALW, website (http://www.cbi.ac.uk/clustalw/). The Molecular Evolutionary Genetic Analysis (MEGA Version 2.1) software was used to estimate nucleotide variability. The genetic distances between the nucleotide sequences from different poultry species were estimated as Kimura 2-parameter distances, using MEGA software. Phylogenetic trees were constructed with Neighbour Joining (NJ) procedure using MEGA Version 2.1. Support of the clusters was evaluated by bootstrap, as percentage recurrence of clusters based on 100 bootstrapped replications with MEGA Version 2.1.

RESULTS AND DISCUSSION

The nucleotide sequence of 571 bp partial CDS of BF2 gene in guinea fowl was compared with standard MHC B haplotypes in chicken. The comparison revealed that the 571 bp fragment (#EU430728) includes 32 nucleotides of signal peptide (exon 1), complete α_1 domain (270 nucleotides) and 269 nucleotides of α_2 domain. The α_2 domain was not complete, but is short of few nucleotides at 3' end. This 571 nucleotide sequence was blasted and the related sequences from other poultry species as well as from mammals were retrieved (Table 1).

In guinea fowl, the α_1 domain was 270 nucleotides in size. It was 6 nucleotides longer in comparison to the α_1 domain in chicken and is due to insertion of 6 nucleotides after 156th nucleotide position. The α_1 domain was also 270 nt long in quails, while in duck and goose, it was 264 nt long. In guinea fowl, the partial CDS of BF2 gene was amplified from 5 randomly selected birds belonging to the closed flock of guinea fowl. No sequence variation was observed between these sequences, suggesting the presence of only one haplotype in this flock. However, high amount of variability in nucleotide sequences were observed within the species (15.93-28.03%) in different poultry species except chicken (7.95%), where it was low (Table 2). Between the guinea fowl and other poultry species, the α_1 domain showed high nucleotide variability ranging from 29.26% (with chicken) to 43.70% (with duck).

In guinea fowl, the α_2 domain was partial and 269 nucleotides in size. In chicken, α_2 domain was 273 nt in size, while in quail, duck and goose, the respective size of α_2 domain was 273 nt, 276 nt and 276 nt. In chicken and quail, 3 deletions were observed after 176 nt position in comparison to guinea fowl, duck as well as goose. Within guinea fowl, no sequence variation was observed in α_2 domain also (Table 2), while high within species nucleotide sequence variability was observed in duck and goose (21.74-22.10), while in chicken and quail, it was comparatively low (9.16 and 13.55, respectively). Between the guinea fowl and other poultry species, high nucleotide variability was observed, ranging from 17.47-37.92%. The α_2 domain showed lesser nucleotide variability as compared to α_1. Earlier worker also found α_1 domain more variable than α_2 domain in chicken (Lima-Rosa *et al.*, 2004) as well as in duck (Moon *et al.*, 2005).

For type of nucleotide substitution, no differences was observed between both the domain as in α_1 ration between synonymous to non-synonymous substitutions was of 1:3.21 and in α_2 domain, this ratio was 1: 3.44. (Table 3). Among various nucleotide substitutions, the several guinea fowl specific substitutions were observed. These substitutions in guinea fowl were at G by A at 3 nt, C by G at 6 nt, G by C at 169 nt, A by G at 170 nt, C by G at 172 nt, G by A at 188 nt, C by A at 205 nt, G by A at 252 nt and A by T at 257 nt in comparison to other poultry species in α_1 domain and were at T by C at 66 nt, A by G at 158 nt, A by G at 215 nt and G by C at 237 in comparison to other poultry species in α_2 domain.

Table 1: Details of the nucleotides sequences of MHC class I gene from different poultry species and mammals used in present study

Accession number	Species	Remark
EO430728	Numida meleagris	Present work
AF013493	Gallus gallus	-
NM_001031338	Gallus gallus	BF2, B21
AY234769	Gallus gallus	BF2*2101
AM282692	Gallus gallus	BF2*0201
AF013492	Gallus gallus	-
Z54326	Gallus gallus	-
AB229813	Coturnix coturnix	MHC Class I, QF41
AB005527	Coturnix coturnix	Coja C
AB078884	Coturnix coturnix	Coja D
AB115246	Anas platyrhynchos	MHC Class I, Du6MHC
AB115241	Anas platyrhynchos	MHC Class I
AB119993	Anas platyrhynchos	MHC Class I
AY294419	Anas platyrhynchos	MHC Class I, Anpl-U*05
AY654899	Anser anser	-
AY387655	Anser anser	-
AM114924	Anser anser	-
AY387651	Anser anser	-
NM_010380	Mus musculis	-
NR_001434	Homo sapiens	-

Table 2: Within as well as between-species percent nucleotide sequence variation in different domains of BF2 gene

	α_1 domain			α_2 domain		
	Polymor-phic sites	Total sites	Percent polymorphism	Polymor-phic sites	Total sites	Percent polymorphism
Within species						
G. fowl	00	270	00.00	00	269	00.00
Chicken	21	264	07.95	25	273	9.16
Quail	43	270	15.93	37	273	13.55
Duck	74	264	28.03	60	276	21.74
Goose	59	264	22.35	61	276	22.10
Between species						
G. Fowl-Chicken	79	270	29.26	47	269	17.47
G. Fowl-quail	79	270	29.26	66	269	24.54
G. Fowl-duck	118	270	43.70	102	269	37.92
G. Fowl-goose	109	270	40.37	98	269	36.43

Table 3: Number of synonymous and non-synonymous substitutions at α_1 and α_2 domain in different poultry species

	α_1 domain		α_2 domain	
Species	Synonymous	Non-Synonymous	Synonymous	Non-Synonymous
Guinea fowl	23.74	76.26	23.37	76.63
Chicken	24.31-24.88	75.13-75.76	23.11-24.28	75.74-76.89
Quail	23.04-23.99	76.01-76.96	21.84-22.86	77.14-78.16
Duck	22.73-24.24	75.76-77.27	21.97-22.35	77.71-78.03
Goose	22.98-23.48	76.52-77.02	22.35-22.86	77.14-77.65
Overall	23.73	76.27	23.02	76.98

Phylogenetic analysis: The genetic distances (Kimura 2-parameter) were estimated using the cumulative nucleotide variability in α_1 domain and α_2 domain (Table 4). Between species genetic distances ranged from 0.186 between duck and goose to 0.401 between quail and duck. Guinea fowl showed lower genetic distances with chicken and quail (0.211-0.215), while with duck and goose, it showed higher genetic distances (0.343-0.350). Duck and goose in general showed high genetic distances with guinea fowl, quail and chicken. Xia *et al.* (2004) also reported much lower homology between the duck and chicken (55.2-64.6%).

Phylogenetic tree, based on pair wise genetic distances estimated on the nucleotide variability in α_1 and α_2 domains (Fig. 1) revealed two major clusters, comprising of guinea fowl, quail and chicken in one, while duck and goose in other. In first cluster, guinea fowl make separate branch, while chicken and

Table 4: Within and between species genetic distance (Poisson correction) based on amino acid diversity in α_1 and α_2 domains

	Guinea fowl	Chicken	Quail	Duck	Goose
Guinea fowl	0.00	0.325	0.327	0.507	0.487
Chicken		0.092	0.307	0.534	0.509
Quail			0.180	0.602	0.564
Duck				0.281	0.308
Goose					0.239

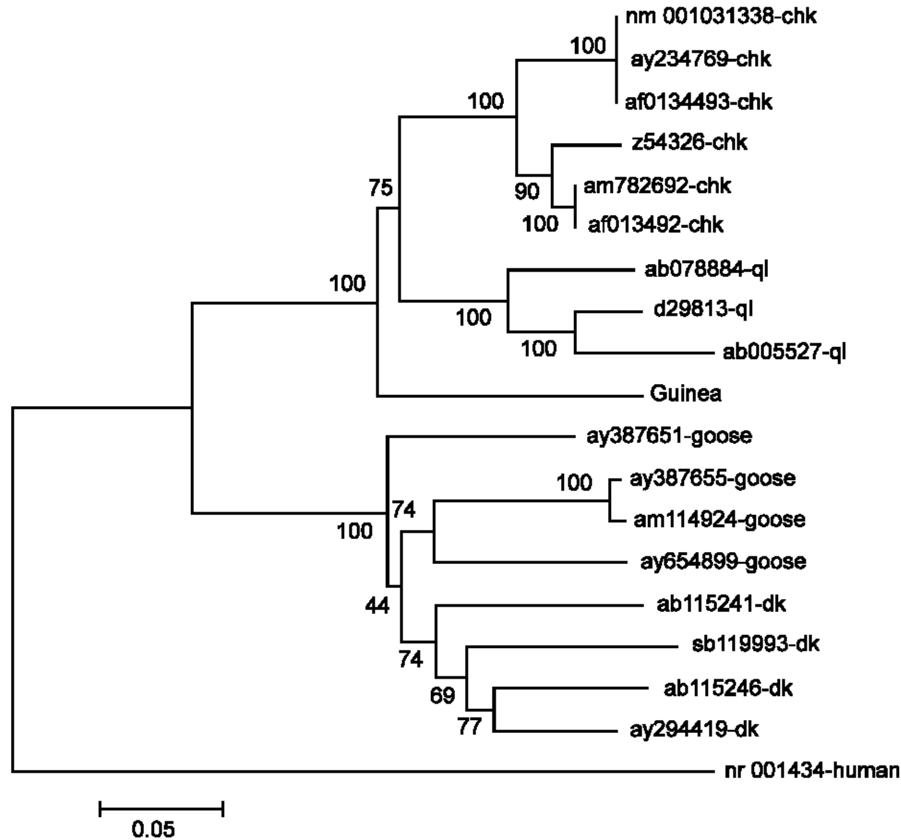

Fig. 1: Phylogenetic tree based on nucleotide variation (A) and amino acid variation (B) in α_1 and α_2 domain of BF2 gene. The phylogenetic tree was outgrouped-rooted by the human sequence. Values at nodes represent bootstrap replication scores (based on 100 resampling)

quails are clustered together. In other clusters, goose and duck cluster together, except one goose sequence, which make a separate branch. In the lineage of MHC class I molecules, guinea fowl showed more closeness to the chicken and quail, while the duck and goose seemed to have a distant lineage. Shiina *et al.* (1999) reported the separate clustering of MHC class I sequences from quail and chicken. Xia *et al.* (2004) found that duck MHC class I clusters quite distantly from chicken MHC.

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