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Full Length Research Paper

Sequence variability in the BLB2 region among guinea fowl and other poultry species

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The BLB2 gene is a predominantly expressed MHC class II gene in chicken and has not been characterized in guinea fowl. In view of the resistance of guinea fowl to most of the chicken diseases, nucleotide sequence variability in β1 and β2 domains of BLB2 gene was studied between guinea fowl and other poultry species such as chicken, quail, pheasant and duck. The β1 and β2 domain of BLB2 gene in guinea fowl was 270 and 282 nucleotides in size and showed no size variation with other poultry species. Between-species percent nucleotide variability ranged from 24.07 to 30.00% in β_1 domain, while in β_2 domain percent nucleotide variability was 8.16 - 10.99%. Between the species, guinea fowl showed low and similar genetic distances (0.149 - 0.159) with all other poultry species, except with duck (0.287). The phylogenetic tree, based on nucleotide variability in β_1 and β_2 domains from these poultry species clearly revealed that duck MHC are clustered separately. Among other poultry species (chicken, quail, pheasant and guinea fowl), the guinea fowl, branched out separately within the cluster comprising of galliformis species.

Key words: BLB2, guinea fowl, nucleotide variability, genetic distance.

INTRODUCTION

Guinea fowl, an important poultry species comes under family Numidae under order galliformes. Guinea fowl differs from the fowl not only in their phenotypic appearance but also in behavioral and production characteristics. One very important characteristics of guinea fowl is its resistance to the common diseases occurring in chicken (Aitken et al., 1977), which make guinea fowl an important model for studying the mechanism of disease resistance.

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 (Sharma, 2007).

The MHC class II genes have not yet been characterized in this poultry species. Hence, an attempt has been made to study the nucleotide sequence variability in the two important domains, that is, β_1 and β_2 of BLB2 gene in guinea fowl and other poultry species.

Major Histocompatability complex (MHC) genes have vital role in influencing resistance to diseases. The MHC class II molecule, an antigen presenting molecule, is comprised of two non-covalently associated polypeptide chains (α chain and β chain). Each chain has two domains (α_1 and α_2 for α chain and β_1 and β_2 for β chain). The peptide-binding region is a groove-like structure which has a floor made by 8 β pleated sheets and two "walls" made by α helices. The β_1 domains make 4 β pleated sheets and one α helix, while α_1 also make 4 β pleated sheets and one α helix. The amino acids facing the groove showed maximum variability. The genetic polymorphism determines the chemical structure of the groove and influences the specificity and affinity of peptide binding and T cell recognition. The immuno-globulin-like region formed by α_2 and β_2 domains is folded into Ig-like domains. These are largely non-polymorphic.

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Abbreviation: MHC, Major histocompatability complex; **nt,** nucleotide.

The correlation of CD4 expression on helper T cells with a specific TCR for class II MHC molecules is due to binding of the CD4 molecules to the Ig-like non-polymorphic β_2 domain of class II molecules. Hence the β_1 and β_2 are important domains of MHC class II region.

The MHC class II region is not characterized in guinea fowl. It is very well studied and characterized in chicken (Bourlet et al., 1988; Zoorob et al., 1990; Sung et al., 1993; Juul-Madsen et al., 2000; Singh et al., 2005), pheasants (Wittzell et al., 1994, 1999), quails (Shiina et al., 2004; Hosomichi et al. 2006) and turkey (Emara et al., 1992; Ahmed et al., 2007). Hence, an attempt has been made to study the nucleotide sequence variability in the two important domains, that is, β_1 and β_2 of BLB2 gene in guinea fowl and other poultry species.

MATERIALS AND METHODS

Sequencing of BLB2 gene in guinea fowl

One healthy guinea fowl bird from a closed flock population of Lavender variety was used. Using two set of primers, that is, set I (5'-GTG CCC GCA GCG TTC TTC-3' and 5'-CAG CTA CGT GTGCCG GGT-3') and set II (5'-GCG GGA GGA GAC GGA GC-3' and 5'-CTC TTC GTG TTC CTG CGC-3') and cDNA as template, 501 bp fragment and 284 bp fragment were amplified and sequenced (EU030445, EU430727, respectively). The 501 bp fragment ((#EU030445) included the complete exon 2, that is, β_1 domain and 231 nucleotides of exon 3, that is β_2 domain, while the 284 bp fragment (#EU430727) was comprised of 163 nucleotides of exon 3, that is, β_2 domain from 3' end, complete exon 4 and 10 nt of exon 5. The complete β_1 and β_2 domains were cut from these sequences and were used for further analysis.

Nucleotide sequence analysis

The related sequences from other poultry species (Figure 1) were retrieved from Genbank (www.ncbi.nlm.nih.gov) and the corresponding regions to β1 and β2 domains were cut and saved. Subsequently, the sequences of β1 and β2 domains from different poultry species were aligned with the respective sequences of β1 and β2 domains from Guinea fowl (present study) separately using CLUSTALW (http://www.cbi.ac.uk/clustalw/). The Molecular Evolutionary Genetic Analysis (MEGA Version 2.1) software was used to estimate nucleotide variability.

The genetic distance between the guinea fowl and different poultry species, based on nucleotide sequence variability in β_1 as well as β_2 domains were estimated as Kimura 2-parameter distance using MEGA software.

Phylogenetic trees were constructed using cumulative nucleotide sequence variability in β_1 and β_2 domains with neighbour joining (NJ) method using MEGA Version 2.1. Support of the clusters was evaluated by bootstrap, as percentage recurrence of clusters based on 1000 bootstrapped replications with MEGA Version 2.1.

RESULTS AND DISCUSSION

Nucleotide sequence variation in BLB2 *gene*

The β_1 domain of BLB2 *gene* in guinea fowl was 270 nt in size. In other poultry species such as chicken, quail, pheasant and duck, the size of β_1 domain was also 270 nt, except one sequence from duck (#DQ490138), where the deletion of 3 nt was observed after 217 nt position. Similarly, β_2 domain of BLB2 *gene* in guinea fowl had 282 nt. In other poultry species, the size of β_2 domains was also 282 nt in size.

Earlier reports also showed no size variation in β_1 as well as β_2 domain in chicken (Zoorob et al., 1990; Sung et al., 1993), in quail (Shimizu et al., 2004; Hosomichi et al., 2006), in pheasant (Wittzell et al., 1999).

In β_1 domain, between-species percent nucleotide variability ranged from 26.67 to 39.35% (Table 1). Most of the nucleotide substitutions were non-synonymous (77.31%). The ratio of synonymous to non-synonymous was 1:3.4.

The β_2 domain showed comparatively lower nucleotide variability as between-species percent nucleotide variability ranged from 8.16 - 20.21% (Table 1). Majority of the nucleotide substitutions in β_2 domain also were non-synonymous (75.11%) with a ratio of 1:3.02 between synonymous and non-synonymous substitutions (Table 1). Xu et al. (1989) also reported higher genetic variability of 14% - 18% in the β_1 domain as compared to the β_2 domain (< 10%) among different haplotypes in chicken. Sung et al. (1993) reported much lower sequence similarities in β_1 domain (72.7 - 92.0%) as compared to that in β_2 domain (87.6 - 100%).

Guinea fowl showed similar nucleotide sequence variability (26.67 - 30.00%) with other galliformes species (Chicken, quail and pheasant) in β_1 domain, whereas with duck, the nucleotide sequence variability was higher (39.35). In β_2 domain, the trend was similar however the magnitude of percent nucleotide variability was low (8.16 to 10.99%) between guinea fowl and other three galliformes species and 20.21% between guinea fowl and duck.

Among different galliformes species similar variability was reported by different workers. Wittzell et al. (1994) reported 82% to 88% similarity in nucleotide sequence between the pheasant and chicken. Jones Dukes et al. (2003) also demonstrated sequence similarity of 84% in turkey BLB2 with chicken and 81% with pheasant. Shiina et al. (2004) also reported an average nucleotide variability of 83.3% between quail and other poultry species. Ahmed et al. (2007) reported the nucleotide sequences of turkey MHC class II loci (β_1 domain) and revealed 89 - 91% similarity with chicken and peacock MHC.

Genetic distances based on sequence variability in β_1 domain and phylogenetic relationships

Between the species, guinea fowl showed similar genetic distances, that is, Kimura 2-parameter using the

Table 1. Percent nucleotide sequence variation in different domains of BLB2 gene between guinea fowl and other poultry species.

	Polymorphic sites	Total sites	% polymorphism	Synonymous	Non-synonymous
β_1 domain					
G. Fowl-Chicken	77	270	28.52	21.21-23.11	76.89-78.79
G. Fowl-Quail	69	270	26.67	21.84-23.86	76.14-78.16
G. Fowl-Pheasant	81	270	30.00	21.97-22.73	77.27-78.03
G. Fowl-Duck	109	270	39.35	22.28-23.99	76.01-77.72
β_2 domain					
G. Fowl-Chicken	27	282	9.57	25.89	74.11
G. Fowl-Quail	31	282	10.99	24.82	75.18
G. Fowl-Pheasant	23	282	8.16	25.53	74.47
G. Fowl-Duck	57	282	20.21	22.10-22.70	77.30-77.90

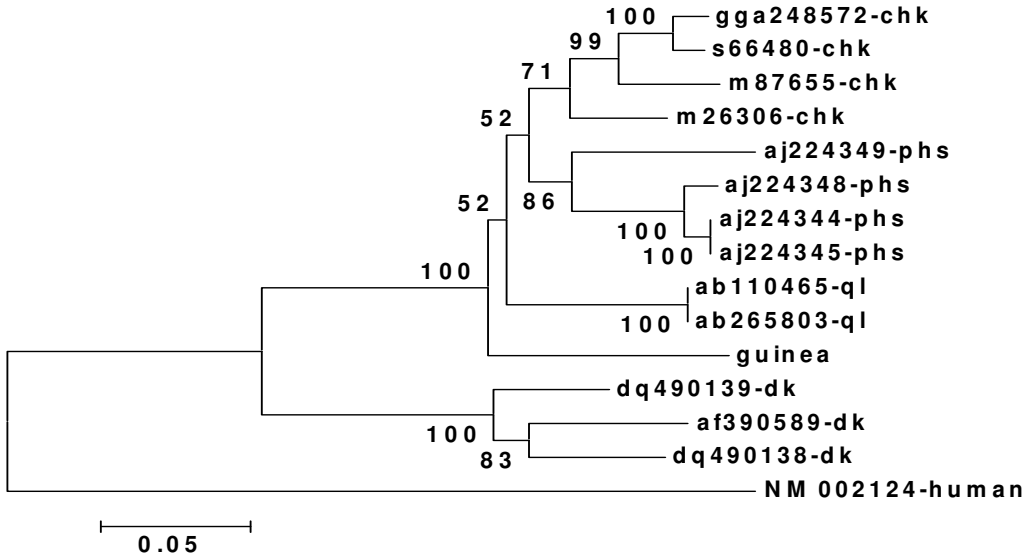

Figure 1. Phylogenetic tree based on nucleotide variation in β1 and β2 domains of BLB2 gene. The phylogenetic tree was outgrouped-rooted by the human sequence. Values at nodes represent bootstrap replication scores (based on 1000 resamplings). GGA248572: Gallus gallus, BLB2 major gene; M26306: Gallus gallus, Clonell-4-1, Haplotype 6, M87655: Gallus gallus, White leghorn BLB2, S66480: Gallus gallus Haplotype 6, AB110465: Coturnix coturnix, CojaII-01HL ; AB265803: Coturnix coturnix, Haplotype 7; AJ224344: Phasianus colchicus, DAB1*0101; AJ224345: Phasianus colchicus, DAB2*0102; AJ224348: Phasianus colchicus, DAB2*04; AJ224349: Phasianus colchicus, DAB1*06; AF390589: Anas platyrhynchos, White pekin, Dq490138: Anas platyrhynchos, DQ490139: Anas platyrhynchos, NM_002124: Homo sapiens.

cumulative nucleotide sequence variability in β_1 and β_2 domains (0.149 - 0.159) with all other poultry species, except with duck (0.287). Duck in general showed high genetic distances with other poultry species (0.275 - 0.289). Singh et al. (2005) reported the genetic in β_1 domain from chicken, quail, pheasant, turkey and duck and reported the estimates ranged from 78.7% (between chicken and turkey) to 55.50% (between quail and duck). While the duck showed much lower genetic similarity with other avian species (55.5 - 61.1%), other poultry species showed higher and similar magnitude of genetic similarity (67.00 - 78.7%) among themselves. The phylogenetic tree based on pair wise genetic distances based on nucleotide variability in β_1 and β_2 domains (Figure 1), clearly revealed that duck MHC are clustered separately, suggesting it distinct and distant relatedness with other

galliformes species. Among other poultry species, chicken and pheasant was sub clustered together, quail emerged as separate branch. The guinea fowl further branched out, though within the first major cluster, but separately from other three poultry species. To study the evolution of avian MHC, Edwards et al. (1999) reported strong clustering of genes within species. Phylogenetic tree of avian MHC genes has been found to be species specific as a result of species specific residues (Takahashi and Nei, 2000). Ahmed et al. (2007) also reported that avian sequences clustered largely by species and the duck MHC made separate branch.

Hence, the β_1 and β_2 domains of BLB2 *gene* showed considerable nucleotide sequence variability between guinea and other poultry species, however β_2 domain showed much lower variability as compared to β_1 domain. For lineage of MHC, guinea fowl MHC class II showed equal distant with the galliformis species (chicken, quail and pheasant), while the duck MHC class II showed very distant lineage as compared to other domestic poultry species including chicken. This distant lineage between duck and other galliformis species such as chicken, quail, turkey, pheasant and guinea fowl is also supported taxonomically as ducks (Order Anseriforms) are separated at order level.

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