INTERNATIONAL JOURNAL OF
IMMUNOGENETICS

doi: 10.1111/j.1744-313X.2012.01126.x

Genetic diversity and population genetic analysis of bovine MHC class II *DRB3.2* locus in three *Bos indicus* cattle breeds of Southern India

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Summary

The present study was performed to evaluate the genetic polymorphism of *BoLA-DRB3.2* locus in Malnad Gidda, Hallikar and Ongole South Indian *Bos indicus* cattle breeds, employing the PCR-RFLP technique. In Malnad Gidda population, 37 *BoLA-DRB3.2* alleles were detected, including one novel allele *DRB3*2503* (GenBank: HM031389) that was observed in the frequency of 1.87%. In Hallikar and Ongole populations, 29 and 21 *BoLA-DRB3.2* alleles were identified, respectively. The frequencies of the most common *BoLA-DRB3.2* alleles (with allele frequency > 5%), in Malnad Gidda population, were *DRB3.2*15* (10.30%), *DRB3*5702* (9.35%), *DRB3.2*16* (8.41%), *DRB3.2*23* (7.01%) and *DRB3.2*09* (5.61%). In Hallikar population, the most common alleles were *DRB3.2*11* (13.00%), *DRB3.2*44* (11.60%), *DRB3.2*31* (10.30%), *DRB3.2*28* (5.48%) and *DRB3.2*51* (5.48%). The most common alleles in Ongole population were *DRB3.2*15* (22.50%), *DRB3.2*06* (20.00%), *DRB3.2*13* (13.30%), *DRB3.2*12* (9.17%) and *DRB3.2*23* (7.50%). A high degree of heterozygosity observed in Malnad Gidda (H_O = 0.934, H_E = 0.955), Hallikar (H_O = 0.931, H_E = 0.943) and Ongole (H_O = 0.800, H_E = 0.878) populations, along with F_{IS} values close to F_{IS} zero (Malnad Gidda: F_{IS} = 0.0221, Hallikar: F_{IS} = 0.0127 and Ongole: F_{IS} = 0.0903), yielded nonsignificant *P*-values with respect to Hardy–Weinberg equilibrium probabilities revealing, no perceptible inbreeding, greater genetic diversity and characteristic population structure being preserved in the three studied cattle populations. The phylogenetic tree constructed based on the frequencies of *BoLA-DRB3.2* alleles observed in 10 *Bos indicus* and *Bos taurus* cattle breeds revealed distinct clustering of specific *Bos indicus* cattle breeds, along with unique genetic differentiation observed among them. The results of this study demonstrated that the *BoLA-DRB3.2* is a highly polymorphic locus, with significant breed-specific genetic diversities being present amongst the three studied cattle breeds. The population genetics and phylogenetic analysis have revealed pivotal information about the population structure and importance of the presently studied three *Bos indicus* cattle breeds as unique animal genetic resources, which have to be conserved for maintaining native cattle genetic diversity.

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Received 20 April 2011; revised 27 February 2012; accepted 30 March 2012

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Introduction

The major histocompatibility complex (MHC) is one of the major components of the immune system. The MHC of cattle is known as bovine lymphocyte antigen (*BoLA*) and is located on the short arm of bovine chromosome 23 (Lewin, 1996). The *BoLA* class II genes encode highly polymorphic αβ heterodimeric transmembrane glycoprotein that bind endocytic-processed antigenic peptides and present themselves on the surface of the antigen-presenting cells (APCs) (Germain, 1994). The interaction between the antigen-MHC class II complex and antigen-specific $CD4^+$ T helper cells activates them, leading to active proliferation of antigen-specific effector T_h and memory T_h cells. Depending on the inflammatory environment and antigenic interactions, effector T_h further differentiate and actively secrete various potent cytokines, chemokines and immunoregulatory proteins (Mosmann & Coffman, 1989; Garcia *et al.*, 1999; Jenkins *et al.*, 2001).

The secreted cytokines stimulate the various stages of B-cell proliferation and differentiation leading to a population of both antibody-secreting plasma cells and memory B cells (Mosmann & Coffman, 1989; Garcia *et al.*, 1999; Jenkins *et al.*, 2001), thus initiating the humoral immune response.

Further, the cytokines secreted by mature $CD4^+$ helper T cells are also necessary for the proliferation of activated $CD8^+$ cytotoxic T cells, thus playing an essential role in antigen-restricted cell-mediated immune response to eliminate altered self-cells and endogenous pathogens (Mosmann & Coffman, 1989; Garcia *et al.*, 1999; Jenkins *et al.*, 2001). The cross-reliance for the activation of $CD8^+$ cytotoxic T cells on $CD4^+$ helper T cells plays a crucial role in preventing T cell-mediated autoimmune response.

The *BoLA-DRB3* is the widely expressed gene in *DRB* locus. It codes for the β chain of the MHC class II heterodimeric transmembrane glycoprotein complex. The exon 2 of the *BoLA-DRB3* gene exhibits a high degree of genetic polymorphism, mainly due to its pivotal role in encoding the antigen binding cleft of the membrane distal, antigen interactive β1 domain of the MHC class II complex (Brown *et al.*, 1993; Russell *et al.*, 1997). Till date, 119 different *BoLA-DRB3.2* allele sequences have been reported in EMBL cattle IPD-MHC database (http://www.ebi.ac.uk/cgi-bin/ipd/mhc/view_nomenclature.cgi?bola.drb3).

Further, the specific *BoLA-DRB3.2* alleles have been associated with resistance to various infectious diseases like mastitis (Sharif *et al.*, 1998; Rupp *et al.*, 2007; Duangjinda *et al.*, 2009), bovine leukaemia virus (Lewin *et al.*, 1999; Juliarena *et al.*, 2008), dermatophilosis (Martinez *et al.*, 2006), immune response to foot and mouth disease (Lewin *et al.*, 1999; Garcia-Briones *et al.*, 2001) and also with milk production traits (Starkenburg *et al.*, 1997; do Nascimento *et al.*, 2006). Therefore, the high degree of polymorphism exhibited by the *BoLA-DRB3.2* gene and its associations with various immunological and milk production traits emphasizes its usefulness as a potential genetic marker in molecular genetics, phylogenetic and marker-assisted selection (MAS) studies of breeds and populations.

India is a vast reservoir of cattle genetic resources, with about 30 recognized *Bos indicus* cattle breeds. These breeds have unique qualities like heat tolerance, ability to withstand environmental stress and resistance to many diseases in harsh tropical climates under low feed-input conditions (Mason, 1996; Nivsarkar *et al.*, 2000).

South India has some of the most important *Bos indicus* cattle breeds, among which Hallikar, Malnad Gidda and Ongole breeds of cattle contribute majorly to draft and dual-purpose *Bos indicus* cattle genetic resources. The Hallikar is a draft purpose *Bos indicus* cattle breed belonging to Mysore type of cattle and distributed in Mysore region and the surrounding districts of Karnataka state (Mason, 1996; Nivsarkar *et al.*, 2000). Malnad Gidda is a small draft *Bos indicus* cattle breed, with compact body frame weighing around 80–120 kg, distributed predominantly in hilly heavy rainfall regions of Malnad and coastal districts of Karnataka (Ramesha *et al.*, 2002; Singh *et al.*, 2008). Ongole is a dual-purpose *Bos indicus* cattle breed mainly found in Nellore, Krishna, Godavari and Guntur districts of Andhra Pradesh (Nivsarkar *et al.*, 2000).

Although previous investigations on genetic polymorphism of *BoLA-DRB3.2* have been investigated in various *Bos indicus* breeds viz, Kankrej cattle (Behl *et al.*, 2007), Brazilian Gir cattle (da Mota *et al.*, 2002), Argentinean Nellore and Gir (Miretti *et al.*, 2001), Hariana, Sahiwal and Rathi cattle breeds (Behl *et al.*, 2009), there are no major previous reports on *BoLA-DRB3.2* locus polymorphism in the above-mentioned three South Indian *Bos indicus* cattle breeds.

Hence, the present study was undertaken with the objective of describing the gene frequency distribution and population genetic analysis of the *BoLA-DRB3.2* locus in Malnad Gidda, Hallikar and Ongole *Bos indicus* cattle breeds. Further, phylogenetic analysis is performed by comparing the gene frequency distributions of *BoLA-DRB3.2* locus observed in the presently studied three *Bos indicus* cattle breeds along with other previously reported *indicus*, *taurine* and *creole* cattle breeds.

Materials and methods

Sampling and DNA extraction

Approximately 10 mL each of whole blood samples were collected in EDTA-coated vacutainer tubes from 107 Malnad Gidda animals situated at Shimoga, Udupi, Kokkada and Nilavara regions of Karnataka state, 73 Hallikar blood samples from Hallikar cattle breeding farm – Kunikenahalli, Bangalore, urban and rural surroundings of Karnataka state – and 60 Ongole blood samples from Ongole cattle breeding farm situated at Chadalavada in Andhra Pradesh state.

Genomic DNA was isolated from peripheral blood lymphocyte by modified salting out method (Miller *et al.*, 1988). The concentration and purity of the DNA were assessed by spectrophotometer and Agarose gel electrophoresis. The DNA samples were diluted and stored at −20°C for further use.

PCR amplification of *BoLA-DRB3.2* gene

A 280 bp *BoLA-DRB3.2* allele-specific PCR product amplification was performed by a single-step PCR amplification reaction utilizing forward primer BL280F1: 5′-ATCCTCTCTCTGCAGCACATT-3′ and reverse primer BL280R2: 5′-CGCTGCACAGTGAA ACTCTC-3′, which were designed based on *BoLA-DRB3.2* gene sequence assigned NCBI GenBank ID: EU586799.

The PCR amplification was carried out in a final volume of 35 μL containing: 1× PCR buffer (10 mM Tris–HCl, 50 mM KCl, pH-8.3), 1.75 mM $MgCl_2$, 100 μM of each dNTPs, 1 Unit Taq DNA polymerase (New England BioLabs, Ipswich, MA, USA), 5 pmol each of BL280F1 and BL280R2 primers (Chromous Biotech, Bangalore, India) and 50 ng of template DNA. The