Identification of BoLA DRB3.2 Alleles Present in White Fulani and Muturu Cattle Breeds

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Abstract

Cattle production is an important aspect of animal agriculture. Disease predisposition in cattle can lead to lowered productivity and poor animal welfare. To select and breed for the fittest cattle population, it is expedient that we understand the genetic basis of disease tolerance/resistance. Major histocompatibility complex (MHC) is a vital component of the immune system in vertebrates. Its genes are crucial determinants for immune response and resistance to infectious diseases. The bovine MHC is referred to as Bovine Lymphocyte Antigen (BoLA) with its most functional and highly variable region located in the exon 2 (BoLA-DRB3.2). Over 100 alleles of BoLA-DRB3.2 have been identified in cattle and many studies have associated polymorphism in this region with disease resistance/susceptibility. In this study, we investigated the polymorphic nature of BoLA-DRB3.2 in the White Fulani and Muturu cattle breeds using a single PCR-sequence based typing. We identified 26 and 25 alleles in White Fulani and Muturu breeds, respectively, with only six alleles being mutual in the two breeds. Some of the alleles identified in this study have been noted as markers for disease status in cattle. BoLA-DRB3*014:01:01, BoLA-DRB3*011:01, and BoLA-DRB3*008:01 alleles have been associated with Bovine leukemia virus (BLV) resistance in cattle. BoLA-DRB3*014:01:01, BoLA-DRB3*011:01, and BoLA-DRB3*008:01 alleles were linked with mastitis resistance in Japanese Holstein cows. While no inference can be drawn in terms of association with disease status, this study confirms the highly polymorphic and diverse nature of BoLA-DRB3 in White Fulani and Muturu cattle breeds.

Keywords

Major Histocompatibility Complex, Bovine Lymphocyte Antigen (BoLA), Polymorphism, White Fulani, Muturu
1. Introduction

Cattle production is a key aspect of livestock agriculture with its output serving as valuable protein source for consumers as well as providing financial benefits for farmers. Challenges posed by diseases can lead to lowered productivity, poor animal welfare and impose huge financial burden on farmers. It is expedient that we understand the genetic basis of disease tolerance/resistance in order to select and breed for the most tolerant or resistant cattle population. Major histocompatibility complex (MHC) is a vital component of the immune system in vertebrates [1]. MHC genes are crucial determinants for immune response and resistance to infectious diseases [2]. The bovine MHC is referred to as Bovine Lymphocyte antigen (BoLA) [3], and it is mapped to BTA 23 [4]. It has two broad divisions—class I and II. The class II region is further sub-divided into class IIa and class IIb [5]. The genes in the MHC class IIa subregion encode either the DQ or DR molecule. DRA genes which encode the α-chain of the DR molecule show less polymorphism in comparison with the gene encoding the β-chain (DRB) [5]. Of the three bovine DRB loci, only DRB3 is functional [6]; and within DRB3, most variation occurs in the exon 2 region [7] [8]. DRB3 is reported to influence the strength and specificity of antigen-specific T-cell response to infection [9]. Polymorphism in the BoLA-DRB3 region has been linked to differences observed in individual cattle in their response to pathogenic diseases [5]. Over 100 alleles of BoLA-DRB3.2 have been reported [10]. As a result of its role in encoding for the β1 domain of the only widely expressed DRB gene in cattle (DRB3) and its high variability, many studies have investigated polymorphism in the BoLA-DRB3 exon 2 (BoLA-DRB3.2), and its association with disease resistance in cattle [11] [12] [13]. In addition to disease status, BoLA-DRB3.2 polymorphism has also been associated with milk production traits [14] [15].

Using PCR-sequence based typing, Takeshima et al. [16] investigated the level of polymorphism and genetic diversity of BoLA-DRB3.2 in Philippine native cattle breeds. They identified 83 BoLA-DRB3.2 alleles in 1120 cattle; with five of the alleles being novel. The researchers also observed that in comparison with breeds from other countries, native Philippine cattle possessed a higher number of alleles (71 vs. 35), making them more polymorphic and diverse. By constructing a phylogenetic tree, the authors concluded that there is a distinction between cattle coming from the northern and southern Philippine. Carignano et al. [17] reported that inter-animal genetic variation in BoLA-DRB3.2 is associated with the level of bovine leukaemia virus infection in Holstein and Holstein x Jersey crossbreed. The outcome of the study showed that DRB3*0902 allele was associated with lower infection load, while two alleles (DRB3*1001 and DRB3*1201) were associated with a high level of infection. Juliarena et al. [13] and Juliarena et al. [18] had earlier associated DRB3.2 alleles with bovine leukaemia virus infection levels but cautioned that, despite high association observed between DRB3*0902 allele and low bovine leukaemia infection level, it is not enough to make a definite conclusion that this genetic variation is solely responsible for the
phenotypic difference observed in cattle. The authors opined that a complex genetic and epigenetic interaction is involved in the regulation of bovine leukaemia virus infection.

There is a dearth of information on the extent of BoLA-DRB3.2 polymorphism in White Fulani and Muturu breeds. The White Fulani, a Bos indicus, is regarded as the most numerous and widespread Nigerian cattle breed representing 37% of the country’s herd while Muturu, a West African taurine, is small-bodied trypanotolerant cattle [19]. The aim of this study was to investigate the polymorphic nature of BoLA-DRB3.2 in the White Fulani and Muturu breeds using a single PCR-sequence based typing.

2. Materials and Methods

Data collection and Animal source
The cattle population used for this study are those described by Ahmed et al. [19]. Briefly, it consisted of 80 cattle, forty from each of White Fulani and Muturu breed reared under a semi-intensive production system. Using the PG-100 collection kit from PERFORMAGENE, nasal swab was collected from each animal for DNA extraction.

Genomic DNA Extraction
Genomic DNA was extracted from the nasal swab as described by Ahmed et al. [19].

PCR amplification and DNA sequencing
280-bp covering all exon 2 of BoLA-DRB3 was amplified in a single PCR [20] using DRB3FWR (CGC TCC TGT GA (C/T) CAG ATC TAT CC) and DRB3REV (CAC CCC CGC GCT CAC C) primers as described by Miltiadou et al. [21]. Primer processing, PCR conditions and protocols are as described by Ahmed et al. [19] except for the 59°C annealing temperature used here. PCR products were checked on 1% agarose gel. PCR amplicon was purified prior to sequencing (removal of contaminants and primers) as described by Ahmed et al. [19]. Sequencing reaction was performed at the IBERS Gogerddan Sequencing Facility primed with the DRB3FRW primer to obtain forward sequences.

Sequence-based typing was used to identify DRB3.2 alleles present in the cattle population examined. BoLA-DRB3.2 allele database, which has all previously identified alleles and sequences, was downloaded from the IPD-MHC website (https://www.ebi.ac.uk/ipd/mhc/group/BoLA). Using Genomic Workbench (CLC Bio Ltd, version 6.0), a custom BLAST database was created for all downloaded BoLA-DRB3 alleles. BLAST analysis of the amplified 280 bp sequence products against the created database was used to detect BoLA-DRB3.2 alleles present in White Fulani and Muturu cattle population. For individual animal sequence searched against the database, BoLA-DRB3.2 allele with the highest percentage identity to the query sequence was selected. Appendix shows the percentage identity to the reference sequence, for all the 78 sequences examined in this study, with averages of 96.74% and 97.48% in White Fulani and Muturu,
respectively. The hit length for each sequence is also included in Appendix.

3. Results

Presented in Table 1 are the BoLA-DRB3.2 alleles identified and their frequencies of occurrence in both breeds. A total of 51 alleles were found (26 for White Fulani and 25 for Muturu). Only six mutual alleles (BoLA-DRB3*048:02, BoLA-DRB3*011:02, BoLA-DRB3*027:08, BoLA-DRB3*028:03, BoLA-DRB3*087:02, BoLA-DRB3*024:16) were identified.

Table 1. Allelic polymorphism of BoLA-DRB3 in White Fulani and Muturu cattle.

| Allele I. D       | Freq. (%) | Allele I. D       | Freq. (%) |
|-------------------|-----------|-------------------|-----------|
| White Fulani      |           | Muturu            |           |
| BoLA-DRB3*048:02  | 5.00      | BoLA-DRB3*048:02  | 2.63      |
| BoLA-DRB3*011:02  | 10.00     | BoLA-DRB3*011:02  | 2.63      |
| BoLA-DRB3*027:08  | 2.50      | BoLA-DRB3*027:08  | 2.63      |
| BoLA-DRB3*028:03  | 7.50      | BoLA-DRB3*028:03  | 5.26      |
| BoLA-DRB3*087:02  | 2.50      | BoLA-DRB3*087:02  | 5.26      |
| BoLA-DRB3*024:16  | 2.50      | BoLA-DRB3*024:16  | 7.89      |
| BoLA-DRB3*083:01  | 2.63      |                   |           |
| BoLA-DRB3*024:02  | 2.63      |                   |           |
| BoLA-DRB3*087:04  | 2.63      |                   |           |
| BoLA-DRB3*011:01  | 10.53     |                   |           |
| BoLA-DRB3*027:10  | 2.63      |                   |           |
| BoLA-DRB3*090:02  | 2.50      | BoLA-DRB3*090:02  | 2.63      |
| BoLA-DRB3*007:02  | 2.50      | BoLA-DRB3*007:02  | 2.63      |
| BoLA-DRB3*100:08  | 2.50      | BoLA-DRB3*100:08  | 2.63      |
| BoLA-DRB3*020:08  | 2.50      | BoLA-DRB3*020:08  | 10.53     |
| BoLA-DRB3*070:01  | 5.00      | BoLA-DRB3*070:01  | 2.63      |
| BoLA-DRB3*064:03  | 5.00      | BoLA-DRB3*064:03  | 2.63      |
| BoLA-DRB3*024:12  | 2.50      | BoLA-DRB3*024:12  | 5.26      |
| BoLA-DRB3*010:02  | 2.50      | BoLA-DRB3*010:02  | 2.63      |
| BoLA-DRB3*077:02  | 2.50      | BoLA-DRB3*077:02  | 2.63      |
| BoLA-DRB3*107:02  | 2.50      | BoLA-DRB3*107:02  | 2.63      |
| BoLA-DRB3*116:02  | 2.50      | BoLA-DRB3*116:02  | 5.26      |
| BoLA-DRB3*097:04  | 2.50      | BoLA-DRB3*097:04  | 2.63      |
| BoLA-DRB3*008:01  | 7.50      | BoLA-DRB3*008:01  | 2.63      |
| BoLA-DRB3*004:01  | 5.00      | BoLA-DRB3*004:01  | 2.63      |
| BoLA-DRB3*097:04  | 2.50      | BoLA-DRB3*097:04  | 2.63      |
| BoLA-DRB3*050:01  | 2.50      | BoLA-DRB3*050:01  | 2.63      |
| BoLA-DRB3*013:04  | 5.00      | BoLA-DRB3*013:04  | 10.53     |
| BoLA-DRB3*024:17  | 2.50      | BoLA-DRB3*024:17  | 5.26      |
| BoLA-DRB3*014:01  | 7.50      | BoLA-DRB3*014:01  | 2.63      |
| BoLA-DRB3*138:01  | 2.50      | BoLA-DRB3*138:01  | 2.63      |
| BoLA-DRB3*087:01  | 2.50      |                   |           |

The boldened first six alleles are mutual between both breeds. Freq. = Frequency.
4. Discussion

**BoLA-DRB3.2 alleles in White Fulani and Muturu breeds**

Fifty-one (51) DRB3.2 alleles were identified in total, with only six mutual alleles (BoLA-DRB3*048:02, BoLA-DRB3*011:02, BoLA-DRB3*027:08, BoLA-DRB3*028:03, BoLA-DRB3*087:02, BoLA-DRB3*024:16) between the breeds. This signifies a high within-breed polymorphism and high level of genetic diversity between the two breeds. None of the mutual alleles have been associated with specific disease status. Genetic diversity in BoLA-DRB3.2 between West African zebu and taurine has been documented. Mikko and Anderson [22] found 13 different BRB3.2 alleles in just 18 cattle of White Fulani and N'Dama breed. Alleles with frequency of 5% and above in this study are BoLA-DRB3*070:01, BoLA-DRB3*014:01:01, BoLA-DRB3*011:02, BoLA-DRB3*028:03, BoLA-DRB3*008:01, BoLA-DRB3*004:01, and BoLA-DRB3*013:04 in White Fulani, and BoLA-DRB3*027:07, BoLA-DRB3*024:16, BoLA-DRB3*021:01, BoLA-DRB3*028:03, BoLA-DRB3*011:01, BoLA-DRB3*087:02, BoLA-DRB3*061:01, and BoLA-DRB3*001:01 in Muturu. These alleles (≥5%) accounted for 55% and 55.25% of the overall allele frequencies in White Fulani and Muturu breeds, respectively.

BoLA-DRB3*070:01, *014:01:01 and *008:01 which had ≥5% occurrence in White Fulani samples of this study were also identified in White Fulani cattle population studied by Mikko and Anderson [22]. Similarly, BoLA-DRB3*001:01, *021:01 and *011:01 that had 5% frequency in Muturu samples in this study were identified in N’Dama breed, an African taurine like Muturu [22]. When compared with DRB3.2 allele frequencies that have been reported in other Bos indicus breeds, BoLA-DRB3*008:01, which is the second most frequently occurring allele in White Fulani population under study (7.5%), was found as the most frequently occurring allele—23.07%, 9.2% and 21.54% in Caracu [23], Saavedreno Creole [24], and Sistani breeds [25] respectively.

Some prominent alleles identified in this study that have been noted as markers for diseases status and those that occurred more frequently are briefly mentioned below. BoLA-DRB3*014:01:01 allele which occurred at a frequency of 7.5% in White Fulani cattle population here, was identified in White Fulani, N’Dama and Swedish Red and White European breed [22]. The allele was also found in Latin American Creole cattle (6.64%), a breed known to have African origin [26]. Takeshima et al. [27] also identified the allele in three South American zebu cattle—Nellore-Brahman (1.54%), Bolivian Holstein (1.57%) and Gir (10%). Takeshima et al. [28] associated BoLA-DRB3*014:01:01 allele with Bovine leukemia virus (BLV) resistance in Japanese Holstein cows. This was based on BLV proviral load—an index for BLV diagnosis—in the cows. Alleles BoLA-DRB3*002:01, *009:02, and *014:01 were identified as resistant alleles, while DRB3*012:01 and *015:01 were associated with bovine leukemia virus susceptibility. The allele (BoLA-DRB3*014:01:01) was also associated with mastitis resistance in Japanese Holstein [29] [30].
BoLA-DRB3*008:01 was found in 7.5% of the White Fulani breed examined. Takeshima et al. [31] identified BoLA-DRB3*008:01 as one of the three most common alleles that accounted for 43.8% of all the alleles identified in 176 Japanese Shorthorn cattle. BoLA-DRB3*009:02 identified in Muturu population under study, with a frequency of 2.63%, was associated with bovine leukaemia virus resistance [17] [28] [32]. The allele suppressed bovine leukaemia virus replication in Japanese Black and Holstein breed in Japan [32].

Gutiérrez et al. [33] reported that BoLA-DRB3*011:01, which was found in Muturu population in this study (10.53% frequency) confers resistance to bovine leukaemia in Harton del Valle cattle breed, with heterozygous individuals responding better to the disease than the homozygous carriers. BoLA-DRB3*001:01 (5.26%) and BoLA-DRB3*011:01 (10.53%) identified in Muturu breed were associated with susceptibility and resistance to mastitis respectively in Japanese Holstein [29] [30].

Peters et al. [34] investigated BoLA-DRB3.2 genetic diversity in 17 cattle breeds from Africa, Asia, and America. Their result showed a higher within breed genetic variation than between breed variation. Three Nigerian breeds (White Fulani, Muturu, and Sokoto Gudali) were included in their study. Sokoto Gudali had 10 haplotypes, which ranked as the highest number of haplotypes observed in a single breed in the study. Muturu and White Fulani had six and eight haplotypes, respectively. This result gave an insight into how polymorphic the Muturu and White Fulani breeds are at the BoLA-DRB3.2 locus.

The allelic information provided in this study would add to the pool of knowledge currently available on DRB3.2 alleles in Nigerian cattle breeds and could also be useful for subsequent association with infectious diseases and immunological traits. It should be noted that the alleles ascribed to each breed here were those with the highest percentage identity with the DRB3.2 sequence for each animal.

A better method to identify BoLA-DRB3 alleles in the highly polymorphic exon two regions would be to use next-generation sequencing, as opposed to Sanger sequencing used here. This would reveal haplotypes and zygosity status for each animal, thereby providing enough confidence to assert the presence of novel alleles.

5. Conclusion

BoLA-DRB3.2 locus appears to be very polymorphic in White Fulani and Muturu cattle breeds. BoLA-DRB3 alleles found in both breeds were identified, some of which have been associated with disease resistance/susceptibility in other cattle breeds. It will be worthy to further explore these alleles in a bid to reveal their status on disease resistance/susceptibility in White Fulani and Muturu cattle.

Authors Contribution

MJ Hegarty supervised the whole process. Ridwan Olawale Ahmed designed the
experiment, carried out laboratory works and wrote the manuscript. Semiu Folaniyi Bello collected field data-collection of nasal swabs. All authors read and approved the manuscript.

**Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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## Appendix. *BoLA-DRB3.2* Alleles, Percentage Identity and Hit Length

| Allele            | %I.D | HL | Allele            | %I.D | HL |
|-------------------|------|----|-------------------|------|----|
| White Fulani      |      |    | Muturu            |      |    |
| BoLA-DRB3*010:02  | 99.63| 273| BoLA-DRB3*027:09  | 99.20| 269|
| BoLA-DRB3*070:01  | 93.63| 270| BoLA-DRB3*011:04  | 96.83| 269|
| BoLA-DRB3*007:02  | 95.17| 272| BoLA-DRB3*020:14  | 97.68| 261|
| BoLA-DRB3*116:02  | 96.55| 263| BoLA-DRB3*024:16  | 97.73| 267|
| BoLA-DRB3*014:01:01| 92.58| 269| BoLA-DRB3*024:16  | 96.63| 270|
| BoLA-DRB3*107:02  | 90.07| 267| BoLA-DRB3*021:01  | 97.20| 269|
| BoLA-DRB3*090:02  | 98.12| 270| BoLA-DRB3*028:03  | 97.39| 276|
| BoLA-DRB3*027:08  | 96.44| 274| BoLA-DRB3*083:01  | 95.85| 268|
| BoLA-DRB3*100:08  | 95.45| 271| BoLA-DRB3*024:02  | 96.67| 270|
| BoLA-DRB3*020:08  | 95.65| 259| BoLA-DRB3*087:04  | 93.50| 271|
| BoLA-DRB3*070:01  | 90.84| 271| BoLA-DRB3*027:07  | 96.80| 271|
| BoLA-DRB3*011:02  | 98.41| 275| BoLA-DRB3*027:07  | 100.00| 274|
| BoLA-DRB3*064:03  | 96.62| 272| BoLA-DRB3*011:01  | 96.24| 268|
| BoLA-DRB3*024:12  | 97.69| 263| BoLA-DRB3*027:10  | 96.50| 260|
| BoLA-DRB3*048:02  | 96.58| 268| BoLA-DRB3*024:16  | 97.75| 270|
| BoLA-DRB3*011:02  | 97.70| 267| BoLA-DRB3*011:05  | 96.98| 268|
| BoLA-DRB3*014:01:01| 99.18| 244| BoLA-DRB3*087:02  | 97.75| 270|
| BoLA-DRB3*020:14  | 97.72| 263| BoLA-DRB3*009:02  | 99.62| 268|
| BoLA-DRB3*024:16  | 98.12| 269| BoLA-DRB3*021:01  | 96.25| 273|
| BoLA-DRB3*028:03  | 98.00| 255| BoLA-DRB3*020:10  | 98.80| 270|
| BoLA-DRB3*028:03  | 97.62| 270| BoLA-DRB3*027:07  | 100.00| 268|
| BoLA-DRB3*097:04  | 97.72| 270| BoLA-DRB3*005:06  | 97.00| 269|
| BoLA-DRB3*028:03  | 96.59| 268| BoLA-DRB3*087:02  | 97.14| 261|
| BoLA-DRB3*011:02  | 97.69| 266| BoLA-DRB3*061:01  | 95.90| 267|
| BoLA-DRB3*011:02  | 97.25| 274| BoLA-DRB3*011:01  | 98.39| 268|
| BoLA-DRB3*008:01  | 97.60| 268| BoLA-DRB3*011:01  | 99.20| 268|
| BoLA-DRB3*008:01  | 97.74| 267| BoLA-DRB3*024:07  | 98.15| 270|
| BoLA-DRB3*004:01  | 97.03| 274| BoLA-DRB3*027:08  | 97.18| 253|
| BoLA-DRB3*048:02  | 98.39| 268| BoLA-DRB3*024:20  | 97.36| 272|
| BoLA-DRB3*014:01:01| 96.46| 266| BoLA-DRB3*011:01  | 98.87| 278|
| BoLA-DRB3*050:01:01| 95.88| 67 | BoLA-DRB3*105:02  | 98.71| 273|
| BoLA-DRB3*013:04  | 98.08| 267| BoLA-DRB3*048:02  | 99.25| 272|
| BoLA-DRB3*024:17  | 97.36| 268| BoLA-DRB3*001:01  | 95.88| 274|
| BoLA-DRB3*013:04  | 98.11| 273| BoLA-DRB3*028:03  | 97.00| 267|
| BoLA-DRB3*087:02  | 96.63| 269| BoLA-DRB3*061:01  | 96.00| 274|
| BoLA-DRB3*064:03  | 96.67| 276| BoLA-DRB3*011:02  | 97.22| 268|
| BoLA-DRB3*004:01  | 97.00| 267| BoLA-DRB3*001:01  | 98.18| 274|
| BoLA-DRB3*008:01  | 96.27| 273| BoLA-DRB3*109:01  | 97.25| 270|
| BoLA-DRB3*138:01  | 97.36| 267| BoLA-DRB3*087:01  | 97.38| 274|

%I.D—% Identity, HL—Hit length.