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*Original scientific paper*

# NUCLEOTIDE SEQUENCE VARIABILITY ANALYSIS OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II DQA1 GENE IN NIGERIAN GOATS

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Major Histocompatibility Complex **(**MHC) molecules loaded with peptides derived from invading pathogens are recognised by the immune system to produce a highly effective and specific response against foreign pathogens. A 310-bp fragment of exon 2 of the MHC Class II DQA1 gene was amplified in 27 animals made up of three major Nigerian goat breeds [West African Dwarf (WAD), Red Sokoto (RS) and Sahel (SH)]. Twenty amino acid polymorphic sites were found in Nigerian goats. Comparison of predicted amino acid residues of DQA1 exon 2 alleles of Nigerian goats with similar alleles from other caprine species revealed considerable congruence in amino acid

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substitution pattern. A significant positive selection signature was detected at the DQA1 locus of Nigerian goats in that non-synonymous substitutions occurred at a faster rate compared to synonymous substitutions (dN:dS ratio = 1.28 ; Z-Statistics= 1.634; P<0.05). The evolutionary tree constructed using UPGMA, revealed that the southern WAD goat appeared to be more related to the northern RS than SH goat at the DQA1 locus. It will be interesting therefore, for future studies to investigate the association of the genetic variants in DQA1 gene of Nigerian goats with resistance/susceptiblity to diseases in order to conserve these precious animal genetic resources.

*Keywords:* DQA1, MHC Class II, Nigerian goats polymorphism, positive selection.

## INTRODUCTION

Genetic variation plays a significant role in maintaining the evolutionary potential of a species. Comparing the patterns of adaptive diversity in extant populations is useful for understanding the local adaptations of a species (CHEN *et al*., 2013). With the developments in molecular biology, researchers can directly examine selection at genes that underlie functional traits. Therefore, adaptive non-neutral markers such as the major histocompatibility complex **(**MHC) genes (NEI *et al*., 2013) have become especially valuable. MHC genes, encoding the MHC molecules are prime candidates for the investigation of genetic variation in the host resistance to infection. These genes have attracted much attention in farm animals due to the need of improved methods of disease control through the design of novel vaccines and selection of disease resistant animals (NIRANJAN *et al*., 2010). In vertebrates, the MHC is vital for foreign antigen recognition and the immune response to infections. Some of its genes are among the most polymorphic loci of the vertebrate genomes displaying high levels of allelic diversity (ALASAAD *et al*., 2012). So far, at least six models have been suggested to explain the maintenance of MHC variability, the two most prominent ones being a) balancing selection and b) the rare allele model. Interestingly, domestic species often have higher than expected levels of MHC diversity, given their domestication history (VILLA *et al*., 2005). The critical role that the MHC plays in the immune recognition of parasites and pathogens renders it evolutionarily relevant in a dynamic fashion to ecology, population biology, and conservation (PIERTNEY and OLIVER, 2006). The MHC class II genes encode polymorphic cell-surface glycoproteins comprising non-covalently linked α and β subunits. DQ genes of MHC class II region encode for α (DQA) and β (DQB) chains of the molecule (NIRANJAN *et al*., 2010). The second exon has been shown to be highly polymorphic and under positive selection, and the class II DQA gene has recently attracted more attention (AMILLS *et al*., 2008; JUNYING *et al.,* 2008; MIYASAKA *et al*., 2011; HOU *et al*., 2011; DENG *et al*., 2013).

In Nigeria and other developing countries, goat production plays an important role in the economic improvement of poor farmers and contributes to poverty alleviation (YAKUBU *et al*., 2011). Although they thrive and adapt to tropical conditions, goats are susceptible to many diseases. The DQ genes have been extensively studied for their allelic variation and haplotypic pattern in most of the farm animals. However, apart from the study of YAKUBU *et al*. (2013) on genetic diversity of MHC DQB1 gene in the three main breeds of goats in Nigeria [West African Dwarf (WAD), Red Sokoto (RS), otherwise known as Savannah brown, and the Sahel (SH) goats], there is dearth of information on any other MHC locus. This lack of genetic characterization in indigenous goats prevents the breeders from implementing genetically-based

selection programmes for improvement of their productivity, immunity, and health. The genetic-based selections are replacing the old phenotypic-based programmes in the developed countries, due to the better advantages in accurate prediction of improvements in production, immunity, and health of the offsprings expected to be produced from the selected animals (GAMA *et al*., 2010).

The present study therefore aimed at characterizing the caprine DQA1 gene as a first step to investigate the existence of association between its genetic variants and resistance /susceptibility to disease pathogens in Nigerian goats.

## MATERIALS AND METHODS

### *Animals and blood collection*

Blood samples (5mL each) were collected from a purposive sample of 315 goats (about 2 years of age) covering various farms, villages and markets in southern (75) and northern (240) Nigeria, sub-saharan Africa (Figure 3.1). Two hundred and twenty samples comprising 80 West African Dwarf (WAD), 90 Red Sokoto (RS) and 50 Sahel (SH) goats yielded high quality DNA. DNA extraction was done using the ZymoBead$^{TM}$ Genomic DNA kit (Zymo Research Corp. Irvine, CA, USA). DNA yield and quality were assessed using Nanodrop ND-100 UV/Vis Spectrophotometer (NanoDrop Technologies, Inc., DE, USA). For DQA1 gene, Genomic DNA from 27 animals comprising 9 each of WAD, RS and SH goats were eventually utilized for polymorphism identification.

### *PCR amplification and sequencing of DQA1 gene*

A 310-bp fragment of exon 2 of DQA1 was amplified in 27 animals for polymorphism identification. Primers were designed using data from AMILLS *et al*. (2005). Primer sequences were DQA/FW, 5′GAAGCCCACAATGTTTGATAGTCA-3′ and DQA/REV, 5′-GGGGAAGAACAACAAAGAGAGGCAG-3′. PCR amplifications were carried out in a C1000$^{TM}$ Thermal Cycler (Bio-Rad, USA) in a total reaction volume of 20µL containing about 20 ng DNA, 10 pmol of each primer in 10 µL Syd Lab PCR Premix (Syd Labs, Inc., Malden, USA) containing Taq DNA polymerase, dNTPs, $MgCl_2$, reaction buffer, PCR stabilizer and enhancer at optimal concentrations. The thermal profile for amplifying the DQA1 exon 2 involved 35 cycles of initial denaturation at 94°C for 4 mins, denaturation at 94°C for 30 s, annealing at 62°C for 30 s, extension at 72°C for 30 s and elongation at 72°C for 10 mins. PCR products were detected on 1.0% agarose. Gels were stained with GelRedR Nucleic Acid Stain (PHENIX Research Product, Candler, NC, USA ) at 5µl/100ml of the agarose gel suspension, scored using GENEMate Quanti-Marker 100 bp DNA ladder (BioExpress, Kaysville, UT, USA), and photographed under UV light. PCR products were sequenced using the same PCR primers with Applied Biosystems Automated 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

### *Sequence analysis*

Nucleotide sequences were edited based on their forward and reverse consensus chromatograms using Codon Code Aligner. Sequence alignments, translations and comparisons were carried out using ClustalW (LARKIN *et al*., 2007). The BLAST algorithm was used to search the NCBI GenBank (http://www.ncbi.nlm.nih.gov/) databases for homologous sequences and to compile a representative number of Nigerian goat sequences (9 sequences) identified in the

present study and Cahi (7), OLA (5), BoLA (5), BuLA (3) and SLA (1) (as an outgroup) DQA1 sequences from GenBank following the method of AMILLS *et al*. (2005) (Table 1).

*Table 1. GenBank accessions for published DQA1 nucleotide sequences*

| Species | No of sequences | Accession numbers |
|---|---|---|
| Goat: *Capra hircus* (Cahi) | 7 | AY464657, AY464654, AY464655, AY464656, AY665664, AY665665 and AY665666 |
| Sheep: *Ovis aries* (OLA) | 5 | Z28418, Z28420, Z28518, AY230210 and AY230208 |
| Cattle: *Bos taurus* (BoLA) | 5 | D50454, AB259567, AB257110, AB257112, AB257113 |
| Buffalo: *Bubalus bubalis* (BuLA) | 3 | DQ440647, DQ116959 and AY954685 |
| Swine: *Sus scrofa* (SLA) | 1 | AY102474 |

The deduced amino sequences of Nigerian goats were compared with published caprine amino acid sequences. The relative proportions of non-synonymous substitutions per non-synonymous site (dN) and the number of synonymous substitutions per synonymous site (dS) were calculated using the method of NEI and GOJOBORI (1986). The ratio of nonsynonymous to synonymous divergence (dN/dS) was tested for departure from the neutral expectation of unity using the codon-based Z-distribution as by modified Nei-Gojobori, applying proportion correction method. The P-value was the probability of rejecting the null hypothesis of positive selection (dN/dS).

Pairwise genetic distances were estimated on the basis of the consensus DQA1 nucleotide sequences of goat, sheep, cattle, buffalo and pig. To test for evidence of trans-species polymorphism, a neighbour-joining tree, depicting phylogenetic relationships among Nigerian goat DQA1 nucleotide sequences and published caprine, ovine, bovine, buffaline and porcine sequences was constructed using the complete deletion and Kimura-2 parameter model options. The reliability of the tree was estimated by bootstrap confidence values (FELSENSTEIN, 1985), with 1000 bootstrap replications, and branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. Similarly, consensus sequence of each of Nigerian goat breed and those of published caprine, ovine, bovine and buffaline sequences were used to obtain a phylogenetic tree based on P-distance model of the UPGMA method of MEGA version 5 (TAMURA *et al*., 2011). The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.5).

## RESULTS

The PCR based amplification of DQA1 region resulted in an amplicon of 310-bp size (Figure 1).

Alignment of deduced amino acid sequences of the DQA1 exon 2 of Nigerian goats and published caprine alleles from the GenBank are shown in Fig. 2. Twenty different polymorphic sites were identified in Nigerian goats which indicate a high degree of polymorphism at the DQA1 locus in this species. There appeared to be more of these allelic variants in SH and RS

goats compared to WAD goats. However, there was a peculiar amino acid substitution (T) found in WAD goats which was absent in SH and RS goats as well as the published caprine sequences.

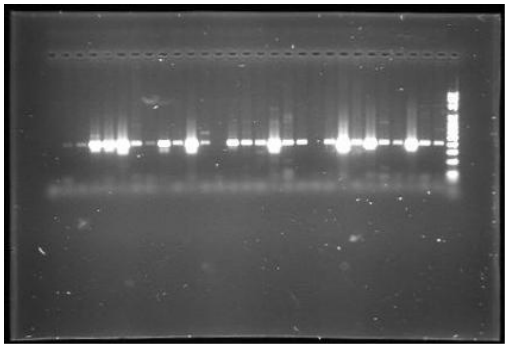

Fig.1. A 310-bp of DQA1 gene in Nigerian goats

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[                    2222333333 3444444444 4555555556 6666666677 7777778888
[                    6789124678 9012345678 9012346891 2345678901 2345681346
#Capra_hircus_DQA1_AY464657.1 CSSPLSPWHL WRKRLPNIWS LWLLYPIWRR VLRGPGKEGD CLASAVIKFP
#Capra_hircus_DQA1_AY464654.1 ...L...CR. .H....L... ..P..... ...R..... ...P...R..
#Capra_hircus_DQA1_AY464655.1 ...L...CR. .H....L... ..P..... ...R..... ...P...R..
#Capra_hircus_DQA1_AY464656.1 ...L..... ..... ..... ..... .....V.
#Capra_hircus_DQA1_AY665664.1 G..... .H.H..... ..... ..... .....
#Capra_hircus_DQA1_AY665665.1 G..... ..... ..... ..... .....
#Capra_hircus_DQA1_AY665666.1 G..... .H.H..... ..... ..... .....
#WAD_DOA1_01                  ..... ..... ..... ..... .....
#WAD_DQA1_02                  ..T..... ..... ..... ..... .....
#RS_DQA1_01                   ...L...CR. .H....L... ..P..... ...R..... ...P...R..
#RS_DQA1_02                   ..... ..... ..... ..... .....
#RS_DQA1_03                   ..... ..... ..... ..... .....
#SH_DQA1_01                   G..... .H.H..... ..... ..... .....
#SH_DQA1_02                   ..... ..... ..... ..... .....
#SH_DQA1_03                   G..... ..... ..... ..... .....
#SH_DQA1_04                   ...L...CR. .H....L... ..P..... ...R..... ...P...R..
[                            11111111 1111111111 1111111]
[                    8889999999 9900000000 0011111111 1222222]
[                    7890123456 7801234567 8901234578 9012346]
#Capra_hircus_DQA1_AY464657.1 SGCTEKHSYG ETFGDLDSKV QLYCCYQQVF TILPLFV
#Capra_hircus_DQA1_AY464654.1 .....GFR .....H.... ..... A.....
#Capra_hircus_DQA1_AY464655.1 .....GVW .....H...L ..... .....
#Capra_hircus_DQA1_AY464656.1 .....CS .....H.... ..... .....
#Capra_hircus_DQA1_AY665664.1 .....HD K...G..... ..... .....
#Capra_hircus_DQA1_AY665665.1 ..... ..... ..... .....
#Capra_hircus_DQA1_AY665666.1 .....HD K...G..... ..... .....
#WAD_DOA1_01                  ..... ..... ..... .....
#WAD_DQA1_02                  ..... ..... ..... .....
#RS_DQA1_01                   .....GVW .....H...L ..... .....
#RS_DQA1_02                   ..... ..... ..... .....
#RS_DQA1_03                   ..... ..... ..... .....
#SH_DQA1_01                   .....HD K...G..... ..... .....
#SH_DQA1_02                   ..... ..... ..... .....
#SH_DQA1_03                   ..... ..... ..... .....
#SH_DQA1_04                   .....GFR .....H.... ..... A.....
```

Fig. 2. Alignment of predicted amino acid sequences of Nigerian goats (WAD, RS and SH) and previously reported caprine MHC-DQA1 exon 2 alleles. All sequences are compared against the consensus. Identity with the consensus is indicated by a hyphen (-).

To identify the signatures of long-term (historical) positive selection, we calculated the rates of nonsynonymous (dN) and synonymous (dS) substitutions (Table not shown). Historical balancing selection was revealed at the DQA1 locus of Nigerian goats, as indicated by excess non-synonymous substitutions (dN:dS ratio = 1.28 ; Z-Statistics= 1.634; P<0.05), thus rejecting the null hypothesis of neutrality.

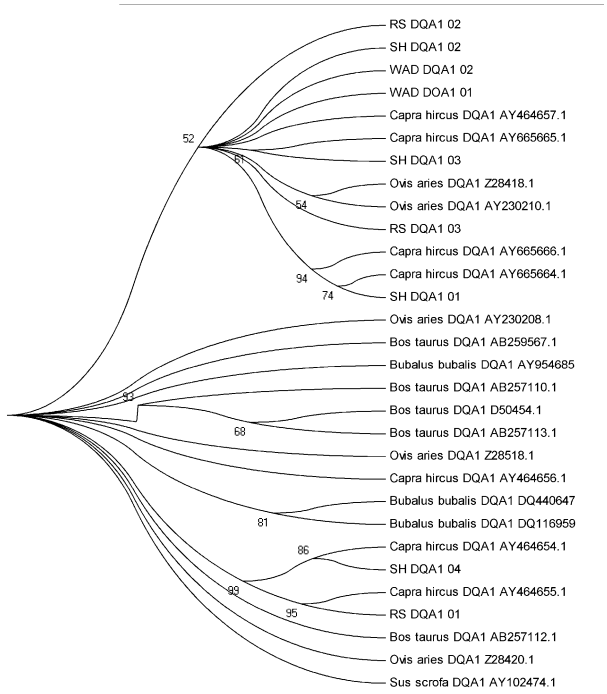


Fig. 3. Neighbour-Joining phylogenetic tree obtained for Nigerian goats DQB alleles and published Caprine, Ovine and BoLA-DQB alleles. The evolutionary history was inferred using the Neighbour-Joining method. Only bootstrap values higher than 50% are shown. The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.5). The tree was rooted with porcine sequence (DQA1AY102474.1).

A phylogenetic tree constructed from exon 2 of the DQA1 sequences of the reported caprine, ovine, bovine, buffaline and porcine sequences revealed that Nigerian goats clustered well with other caprine sequences (Fig. 3). However, there was intermingling among some of the DQA1 alleles of the various species, depicting trans-species polymorphism. The evolutionary history inferred using UPGMA, revealed that all the goat sequences (Nigerian goats and published caprine sequence) clustered together (Fig. 4). The ovine sequence was closer to those of goats compared to the large ruminants (bovine and buffaline sequences). However, the southern WAD goat appeared to be more related to the northern RS than SH goat at the DQA1 locus.

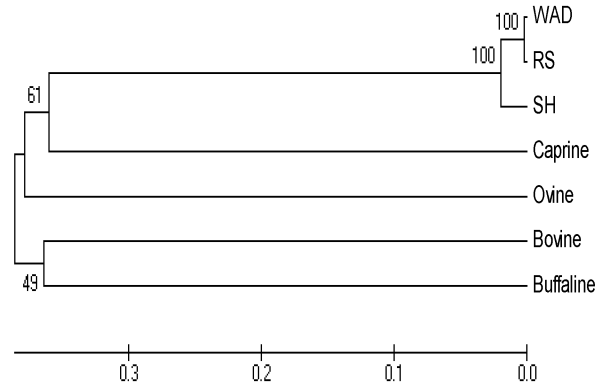


Fig. 4. Phylogenetic tree derived from consensus sequences of each of Nigerian goat breeds (WAD, RS and SH) and those of published caprine, ovine, bovine and buffaline DQA alleles using the UPGMA method.The optimal tree with the sum of branch length = 1.89524254 is shown.

## DISCUSSION

It may be possible to determine the value of maintaining particular genetic variants in a population because of their adaptive significance. The MHC, which is one of the most polymorphic regions in vertebrates, plays significant roles in adaptive immunity. However, this is the first time adaptive variations at MHC DQA1 locus have been investigated in extant Nigerian goat populations. Comparison of predicted amino acid residues of DQA1 exon 2 alleles of Nigerian goats with similar alleles from other caprine species revealed considerable congruence in amino acid substitution pattern in the present study. The preservation of few but highly divergent and functional MHC alleles could be interpreted as balancing selection shaping MHC diversity in the current population (EJSMOND and RADWAN, 2009). This pattern is consistent with the mechanism of divergent allele advantage (CASTRO-PRIETO *et al*., 2011) because high divergence among alleles can result in a wider array of pathogen-derived antigens being recognized by the host population. MHC variability is believed to determine the capability of individuals to resist continuously evolving pathogens and parasites. Consequently, MHC variability is a reflection of the processes that are related to adaptive evolution within and between populations (SPURGIN *et al*., 2010). Thus most variation at MHC loci reflects the effects of balancing selection which is the main mechanism for retaining high MHC genetic diversity (ALCAIDE, 2010). Therefore, priority could be given to breeding individuals or groups of individuals that would maintain these adaptive variants.

It is generally accepted that the polymorphism of MHC genes is most likely maintained by positive selection and that such selection usually results in more non-synonymous than synonymous changes in the codons (WU *et al*., 2012). In the present study, a significant selection signature was detected at the DQA1 locus in that non-synonymous substitutions occurred at a faster rate compared to synonymous substitutions. As MHC genes are subject to selective pressures (LUO *et al*., 2012), the maintenance of genetic variation is of particular interest. Balancing selection includes frequency-dependent selection, overdominance and diversifying

selection and promotes long evolutionary persistence of individual alleles and strongly differentiated allelic lineages in mammals (SOMMER, 2005). The evidence for positive selection at DQA1 in this study is consistent with findings for class II genes of other mammalian species (AMILLS *et al*., 2008; ČÍŽKOVÁ *et al*., 2011).

In the present study, evolutionary relationships of DQA1 alleles revealed phylogenetic patterns similar to those previously described for cattle, bison, sheep and goats (ZHOU and HICKFORD, 2004, AMILLS *et al*., 2004, 2005; TAKESHIMA *et al*., 2008), which strongly supports the existence of trans-species polymorphism in the Bovidae. Trans-species polymorphism, where similar alleles are found in related species due to the passage of alleles from ancestral to descendant species, is hypothesized to be maintained by balancing selection (KLEIN *et al*., 1998). According to YEAGER *et al*. (1999), one of the features of the MHC given its extensive polymorphism, is the presence of shared residues and motifs among loci within the same species as well as between distantly related species.

## CONCLUSION

The study revealed that twenty amino acid polymorphic sites were found in Nigerian goats. The polymorphism of MHC Class II DQA1 Gene in Nigerian goats was driven by a balancing selection mechanism as represented by higher value of dN:dS ratio. The southern WAD goat appeared to be more related to the northern RS than SH goat at the DQA1 locus These results may help to provide more detailed genetic information for conservation and especially health management strategies geared towards improved livestock breeding in the future in Nigeria, sub-saharan Africa and other developing nations.

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## ANALIZA VARIJABILNOSTI NUKLEOTIDNIH SEKVENCI MAJOR GENA HISTOKOMPATABILNOG KOMPLEKSA KLASA II DQA1 KOD NIGERIJSKIH KOZA

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Izvod

*Major Histocompatibility Complex (MHC)* molekule unete sa peptidima patogena imuni sistem prepoznaje i odgovara veoma efikasno i specifično na njih. Fragment od 310-bp egzona 2 MHC klase II DQA1 gena umnožen kod 27 životinja je dobijen od tri glavne nigerijske koze (West African Dwarf (WAD), Red Sokoto (RS) i Sahel (SH)). Utvrđeno je 20 aminokiselinskih polimorfnih mesta. Poređenjem alela nigerijskih koza sa sličnim alelima ostalih kičmenjaka dobijena je značajna podudarnost. Filogenetsko stablo konstruisano korišćenjem UPGMA, ukazalo je da je južna WAD rasa koze, bliskija sa severnom rasom RS, nego sa SH, na lokusu DQA1. Zbog toga će biti interesantno ispitivati vezu između genetičkih varijanti DQA1 gena nigerijske koze i otpornosti/osetljivosti na bolesti, u cilju konzervacije ovih genetičkih resursa.

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