IDENTIFICATION OF SNPs IN THE EXON 2 REGION OF MHC CLASS II GENE (BoLA DR-ALPHA) IN WHITE FULANI AND ITS HYBRID

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ABSTRACT

This study characterized single nucleotide polymorphisms (SNPs) within exon 2 of the BoLA-DR-ALPHA gene across White Fulani cattle (Bos indicus) and their hybrid counterparts in Nigeria. As a critical component of the Major Histocompatibility Complex (MHC) class II pathway, BoLA-DR-ALPHA facilitates antigen presentation and influences disease resilience. Through bidirectional Sanger sequencing of 30 genomic samples (15 pure White Fulani, 15 White Fulani × Girolando/Holstein hybrids), we identified 23 SNPs, including 14 non-synonymous variants. Hybrid populations exhibited 56% greater SNP diversity alongside elevated heterozygosity values (0.68 versus 0.45 in purebreds). Phylogenetic reconstruction revealed distinct clustering of White Fulani with Bos taurus, whereas hybrids demonstrated genetic introgression from Sokoto Gudali lineages. Computational predictions identified five non-synonymous SNPs with potentially deleterious impacts on antigen-binding function. These findings illuminate the role of BoLA-DR-ALPHA diversity in tropical adaptation and inform strategies for preserving disease resilience within crossbreeding initiatives.

Keywords: BoLA-DR- ALPHA, SNPs, MHC, Exon 2, Sanger sequencing, PCR amplification

DESCRIPTION OF PROBLEM

The Major Histocompatibility Complex (MHC) coordinates adaptive immunity through antigen presentation mechanisms. In bovines, the BoLA-DR-ALPHA gene (chromosome 23) encodes the α-chain subunit of MHC class II heterodimers, which bind extracellular pathogen-derived peptides for CD4+ T-lymphocyte recognition [1]. While BoLA-DRB3 polymorphism has been extensively documented, DRA remains inadequately characterized in African *Bos indicus* breeds despite its fundamental role in peptide binding specificity [2]. White Fulani cattle represent Nigeria's predominant indigenous breed, constituting approximately 37% of the national herd. This breed exhibits exceptional trypanotolerance and thermotolerance adaptations [3]. Contemporary breeding programs increasingly utilize crossbreeding with exotic dairy breeds such as Girolando and Holstein-Friesian to enhance productivity. However, this practice risks diluting locally adaptive alleles [4]. The exon 2 region of BoLA-DR-ALPHA encodes the polymorphic α1 domain that forms the peptide-binding groove, rendering it a strategic target for genetic variation analysis. This research addresses three critical knowledge gaps: the scarcity of BoLA-DR-ALPHA diversity data in West African cattle populations; the genetic consequences of hybridization on immunologically relevant loci; and the functional implications of exon 2 polymorphisms on disease resilience mechanisms.

MATERIALS AND METHODS

Blood Sample Collection DNA Extraction

Venous blood samples were collected from two cohorts: fifteen pure White Fulani individuals from pastoral production systems in Osun State, Nigeria; and fifteen hybrid animals (White Fulani × Girolando or Holstein-Friesian) from semi-intensive farms in Oyo State, Nigeria. Genomic DNA was isolated from EDTA-anticoagulated blood using commercial extraction kits (Zymo Research), with purity and concentration verified spectrophotometrically (NanoDrop™, $A_{260/280}$ > 1.8).

Target Amplification and Nucleotide Sequencing

The exon 2 region of BoLA-DR-ALPHA was amplified via polymerase chain reaction using established primers. Thermocycling conditions comprised initial denaturation at 94°C for 300 seconds, followed by 35 amplification cycles (94°C for 30 seconds, 53°C for 60 seconds, 68°C for 90 seconds), and final extension at 68°C for 600 seconds. Purified amplicons underwent Sanger sequencing (ABI 3500XL platform).

Bioinformatics

Sequence chromatograms were quality-trimmed and assembled using BioEdit v7.2.5. Multiple sequence alignment against the *Bos taurus* reference genome (ARS-UCD1.2) was performed with ClustalW in MEGA11. Polymorphic site identification and haplotype reconstruction utilized DnaSP v6.12.03 with a minimum quality threshold of Q30. Non-synonymous substitutions underwent functional impact assessment via SIFT and PROVEAN algorithms. Population genetic metrics—including observed heterozygosity (H_o), expected heterozygosity (H_e), and nucleotide diversity (π)—were computed within DnaSP.

RESULTS AND DISCUSSIONS

Polymorphism Distribution and Functional Consequences

Comprehensive analysis identified 23 validated SNPs within the BoLA-DR-ALPHA exon 2 region. Among these, 14 polymorphisms (60.9%) were non-synonymous substitutions inducing amino acid alterations at 12 residue positions, while 9 variants (39.1%) represented synonymous changes. Three non-synonymous SNPs were absent from public databases (dbSNP), suggesting novel breed-specific variations.

Notable group-specific patterns emerged: hybrid populations harbored 18 SNPs with 9 unique variants, while pure White Fulani cattle exhibited 12 SNPs including 3 unique polymorphisms. Eleven SNPs were conserved across both cohorts. Table 1 summarizes key non-synonymous variants and their computational functional predictions.

Comparative Genetic Diversity Metrics

Hybrid populations demonstrated significantly enhanced genetic diversity relative to pure White Fulani. Observed heterozygosity reached 0.68 in hybrids compared to 0.45 in purebreds. Similarly, nucleotide diversity (π) was elevated in hybrids (0.021) versus purebreds (0.009). Haplotype analysis revealed greater allelic richness in hybrids (9 haplotypes) relative to pure White Fulani (4 haplotypes). Table 2 provides comprehensive diversity indices.

Phylogenetic Relationships and Haplotype Architecture

Phylogenetic reconstruction positioned White Fulani sequences within the *Bos taurus* clade (98.94% identity) but revealed a distinct sub-branch, suggesting conserved adaptive evolution. Conversely, hybrid sequences exhibited closer affinity to Sokoto Gudali lineages than to parental White Fulani haplotypes, indicating substantial genetic introgression. Median-joining haplotype networks demonstrated elongated branching in White Fulani (e.g., RW1↔DWF6: 8 mutational steps), contrasting with complex reticulated networks in hybrids (e.g., DHY5↔BH7: 12 mutational steps with multiple intermediates).

Functional Implications of Non-Synonymous Variants

The substantial frequency of non-synonymous SNPs (60.9%) challenges historical assumptions regarding DRA sequence conservation in bovines. Five variants—notably G274A within a β-sheet substrate—were computationally predicted to disrupt peptide-binding affinity. This observation parallels documented BoLA-DQA2 polymorphisms associated with mastitis resistance [5], suggesting broader functional significance across MHC class II loci. White Fulani-specific substitutions (e.g., C212T) may contribute to trypanotolerance phenotypes, warranting experimental validation through pathogen challenge studies [6].

Hybrid-Derived Diversity and Conservation Challenges

Hybrid populations demonstrated substantially elevated genetic diversity metrics, consistent with heterosis phenomena. However, phylogenetic displacement toward Sokoto Gudali alleles

indicates dilution of White Fulani-specific immunological adaptations. This mirrors observations in Brazilian Girolando populations, where Holstein introgression diminished innate tick resistance [7]. Strategic crossbreeding programs should therefore prioritize marker-assisted conservation of critical immune loci while selectively introgressing productivity traits [8]. The restricted haplotype diversity observed in pure White Fulani (n=4) signals vulnerability to genetic erosion [9].

Table 1: Functional Characterization of Select Non-Synonymous SNPs

Genomic Position	Nucleotide Substitution	Amino Acid Change	Predicted Functional Consequence	Population Distribution
274	G→A	Glycine→Serine	Damaging (SIFT score 0.02)	White Fulani and Hybrid
301	A→G	Lysine→Arginine	Deleterious (PROVEAN score -3.1)	Hybrid-specific
212	C→T	Threonine→Isoleucine	Tolerated (SIFT score 0.35)	White Fulani-specific

Table 2: Population Genetic Diversity Parameters

Breed	Observed Heterozygosity (H_o)	Expected Heterozygosity (H_e)	Nucleotide Diversity (π)	Haplotype Count
Pure White Fulani	0.45	0.51	0.009	4
Hybrid	0.68	0.74	0.021	9

Conclusion and Application

This investigation provides novel insights into BoLA-DR-ALPHA genetic architecture within Nigerian cattle populations. Key conclusions indicate that hybridization enhances immunogenetic diversity but risks diluting locally adaptive alleles; five non-synonymous SNPs exhibit potential to disrupt antigen presentation pathways; and White Fulani cattle maintain phylogenetically distinct haplotypes warranting conservation prioritization.

Nigeria's National Livestock Transformation Plan should consequently implement three key initiatives: establishment of genotype-informed breeding protocols to preserve BoLA-DR-ALPHA/DRB3 haplotypes; development of cryopreserved germplasm repositories for pure lineages; and supervised marker-assisted introgression of resilience traits into commercial hybrids. Study limitations include sample size constraints and absence of phenotypic disease resistance data, necessitating future correlation studies. Three evidence-based recommendations emerge: first, incorporate BoLA-DR-ALPHA variants into marker-assisted selection programs to balance productivity and resilience; second, establish national breed-specific allele frequency databases; third, investigate SNP-disease associations through controlled pathogen challenge trials. Systematic BoLA-DR-ALPHA profiling offers a viable pathway to optimize sustainability within Nigeria's evolving livestock sector.

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