Nig. J. Biotech. Vol. 36 (2) : 150 –157 (Dec 2019)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and www.biotechsocietynigeria.org
DOI: https://dx.doi.org/10.4314/njb.v36i2.15

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Evidence of purifying selection in exon 3 of interferon regulatory factor-5 (IRF-5) gene in Nigerian indigenous chickens

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Abstract

Immune genes are under acute selective pressure in order to resist pathogenic attacks. It is not really clear the type of selective force that acts on immune genes because of diverse pathogen load and host population density, so this experiment studied the selective force acting on exon 3 of IRF-5 gene in Nigerian indigenous chickens. DNA was extracted from 90 Nigerian indigenous chickens and exon 3 of IRF-5 gene was sequenced. The region was tested for deviation from neutrality using DnaSP. The Mean non-synonymous substitutions per non-synonymous site (dN) and mean synonymous substitutions per synonymous site (dS) were calculated to predict likely selective force/event acting on the region using HyPhy software implemented inside MEGA6 software. All the test of neutrality indices obtained for exon 3 of IRF-5 gene in Nigerian indigenous chickens were greater than 1 except Tajima's D value of normal feather chickens (0.93) and Fu's Fs value of naked neck chickens (0.71). The dN of 0.00 and negative dS were estimated for exon 3 of IRF-5 gene in all the three genotypes. This study therefore concluded that purifying selective forces are acting on exon 3 of IRF-5 gene in Nigerian indigenous chickens.

Keywords: Chickens, co-evolution, immunity, pathogens, selection.
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Introduction

Indigenous chickens have evolved through adaptation to various agro-climatic conditions. They possess gene combinations and special adaptation not found in other improved modern breeds (Egahi et al., 2010). They are found in all developing countries and play a vital role in many poor rural households (Alexander et al., 2004). They supply uncommon animal protein in the form of meat and egg which are sold to meet basic family needs such as medicine, clothes and payment of school fees (Bagnol, 2001). Indigenous chickens are usually owned and managed by women in Africa, so they are important components of female-headed households (Gueye, 2000; Bagnol, 2001). They also have a natural scavenging and nesting behaviour. Many decades of natural selection and scavenging conditions, have made them vigorous and tolerant/resistant to various diseases, parasites and stress. They possess a better survival rate than commercial genotypes under local production environments (Minga et al., 2004).

The host defence system (immunity) is categorised into non-specific or innate immunity and specific immunity. Host immune response represents a highly regulated yet integrated interaction between different types of cells that respond to eliminate foreign invaders (pathogens) (Pal et al., 2011). It is the expression of the self for its own wellbeing carried out via an array of several interacting

molecules. Interferon regulatory factor molecules are one of such molecules, conferring self-defence to the host against various pathogens. They are mostly found on various differentiated cell types present in the body (Tamura et al., 2008).

The interferon regulatory factors are transcription mediators of virus-, bacteria- and interferon-induced signalling pathways and so they play a crucial role in antiviral defence, immune response and cell growth regulation (Barnes et al., 2002). Notable homology in the N-terminal amino acid residues are shared among all interferon regulatory factors with DNA-binding domain characterised by IRF tryptophan pentad repeats. Three of these repeats recognise only the GAAA and AANNNGAA sequences present in interferons (Escalante et al., 1998). The function of each IRF is dictated by a combination of cell-type-specific expression, its transactivation potential and its ability to interact with other IRFs (Taniguchi et al., 2001).

The IRF-5 is implicated in immune response to pathogens and apoptosis. It is also implicated in the innate inflammatory responses (Takaoka et al., 2005; Stein et al., 2018). It is distinct from IRFs-3 and 7 by containing two nuclear localization signals with one present in IRFs-3 and 7. Activation and phosphorylation of IRF-5 by viral infection can be detected in cells infected with ND virus and vesicular stomatitis virus but not in cells infected with Sendai virus which is an indication of virus specific activation (Barnes et al., 2002). Type I interferons such as IFN-A, IFN-B and IFN-G; and viral infection stimulate expression of IRF-5 gene (Mancl et al., 2005). It can also be induced by tumour suppressor, p53 (Mori et al., 2002). The gene stimulates cyclin-dependent kinase inhibitor p21 and represses cyclin B1. It also stimulates the expression of proapoptotic genes such as Bak1, Bax, Caspase 8 and DAP kinase 2 (Barnes et al., 2002). It also promotes cell cycle arrest and apoptosis independently of p53 (Hu and Barnes, 2006). Over-expression of IRF-5 gene up-regulates many early inflammatory genes such as RANTES, I-309, MCP-1 and IL-8, which is an indication that IRF-5 has an important role in the transcriptional regulation of the early inflammatory cytokines and chemokines (Barnes et al., 2004; Esmaeili Reykande et al., 2018). There is also a link between IRF-5 expression, IFN-A production and autoimmunity as mutations in IRF-5 gene confers predisposition to systemic lupus erythromatosis which is an autoimmune disease characterised by constitutive IFN-A production (Graham et al., 2006).

Genes implicated in immunity are under acute selective pressure in order to resist pathogenic attacks (Downing et al., 2009). There exist two contrasting views about selective forces acting on immune genes. The first view states that immunity is ancient and important, then selection had sufficient time to fix the most effective alleles and as such there is no tolerance for new alleles, so purifying selection or negative selection is major selective force acting on immune genes (Mukherjee et al., 2009). The other view states that given the rapid rate of pathogen evolution, selection for high allelic diversity increases the flexibility and broad spectrum anti-pathogenic activity that characterises the immune system, therefore, diversifying or positive selection are the main selective drivers (Ferrer-Admetlla et al., 2008).

It is not really clear the type of selective force that act on immune genes because of diverse pathogen load and host population density, so this experiment studied the selective force acting on exon 3 of IRF-5 gene in Nigerian indigenous chickens

Materials and Methods

Experimental site

The experiment was carried out at the Poultry Breeding Unit of the Directorate of University Farms, Federal University of Agriculture, Abeokuta, Alabata, Ogun State, Nigeria. Alabata (latitude $7^{0}10'$N and longitude $3^{0}2'$E) is located in Odeda Local Government Area of Ogun State, Nigeria. The area which lies in the South Western part of Nigeria has a prevailing tropical climate with a mean annual rainfall of about 1037 mm. The mean ambient temperature ranges from 28^{o}C in December to 36^{o}C in February with a yearly average humidity of about 82%. The vegetation represents an interphase between the tropical rainforest and the derived savannah (Google Earth, 2018)

Source, sample size and management of experimental birds

The experimental birds were generated from mating of parent stocks of indigenous chickens

available on the farm through artificial insemination. Ninety birds (27 Normal Feather, 45 Naked Neck and 18 Frizzle Feather chickens) were used for the experiment. The experimental birds were raised under intensive management system. The chicks were brooded in deep litter pen at the brooding stage. The birds were wing-tagged for proper identification. They were also subjected to the same management procedures throughout the experimental period. Commercial feeds were provided for the turkey birds without restriction. Chick starter mash containing 23% crude protein and 11.1MJ/kg metabolizable energy was fed to the birds from 0 to 8 weeks of age. Grower mash containing 18% crude protein and 10.48MJ/kg metabolizable energy was fed to the birds from 9-20 weeks of age. Potable water was provided for the birds without restriction. Vaccination schedule for chicken was strictly adhered to and adequate sanitation was practised to prevent occurrence of diseases.

Blood collection and DNA extraction

About 1 ml of blood was collected from brachial vein of each bird using needle and syringe. The blood was deposited in ethylene diamine tetra acetic acid (EDTA) bottle. Genomic DNA was extracted at Biotechnology laboratory of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta from the birds using Zymo research quick-gDNA^{TM} miniprep kit (catalogue number: D3024) following the manufacturer's protocols.

DNA quantification

The extracted gDNA was quantified for concentration and purity using Nanodrop spectrophotometer using the protocol described by Desjardins and Conklin (2010). The integrity of the gDNA was also checked using gel electrophoretic method by running 1 µl of each gDNA sample on 1.5% agarose gel at 120 V for 20 minutes.

Amplification and sequencing of exon 3 of IRF-5 gene in Nigerian indigenous chickens

Polymerase chain reaction (PCR) was carried out using designed Fwd 5'-TAACCACAACCCAATGATGC-3' and Rev 5'-ATTCCCCCATAAAACACCC-3' primers to amplify 742 bp region covering parts of intron 2 and exons 3-5**.** For amplification, 1 µl of genomic DNA (~10-15 ng) was added to a reaction mixture containing 16.8 µl of nuclease free water, 2.5 µl of 10× PCR buffer, 1.5 µl of 25 mM $MgCl_2$, 1 µl of 5 mM dNTP, 1 µl of 10 UM forward primer, 1 µl of 10 UM reverse primer and 0.2 µl of 10 U/µl surf Hot Taq. The PCR conditions included initial denaturation at 96^0C for 15 minutes, 35 cycles of final denaturation at 95^0C for 30 seconds, annealing at 62^0C for 30 seconds, extension at 70^0C for 1 minute and final extension at 70^0C for 5 minutes.

The amplicon was purified with Magnetic Beads Carboxylate (MCLab, USA). Sequencing of PCR products was done using BigDye Terminator v. 3.1 using the instrument 3730 XL following the supplier's protocol at STAB VIDA, Investigacao e Servicos em Ciencias Biologicas Lda, Caparica, Portugal.

Trimming and cleaning of sequences

The nucleotide sequences were trimmed and edited using Bioedit and MEGA 6 software to remove noises in the sequences.

Multiple sequence alignment

The sequences obtained for exon 3 were aligned with reference exon 3 (NM001031587.1). The alignment was carried out on all the nucleotide sequences using Clustal W software (Thompson et al., 1994) incorporated inside MEGA 6 software.

Identification of single nucleotide polymorphisms

The SNPs present in exon 3 of IRF-5 gene in Nigerian indigenous chickens were identified by aligning each exon with the reference exon downloaded from Ensembl database using Clustal W (Thompson et al., 1994). The SNPs were also confirmed using DnaSP (Librado and Rozas, 2009).

Selection analysis

Tajima's D, Fu's Fs, Fu, Li's D* and Fu and Li's F* tests were performed to test exon 3 of IRF-5 gene in Nigerian indigenous chickens deviation from neutrality using DnaSP (Librado and Rozas, 2009).

Mean non-synonymous substitutions per non-synonymous site (dN) and mean synonymous substitutions per synonymous site (dS) were calculated for exon 3 of IRF-5 gene in Nigerian indigenous chickens to predict likely selective force acting on the exon using HyPhy software implemented inside MEGA6 software. Positive dN-dS value suggests positive selection while negative dN-dS suggest negative selection.

Results

Polymorphisms identified in exon 3 of IRF-5 gene in Nigerian indigenous chickens

Four single nucleotide polymorphisms, 33A>G (rs317511101), 48G>A (rs312902332), 57T>C (rs315149141) and 174T>C (rs739389464), were identified in exon 3 of interferon regulatory factor-5 gene in Nigerian indigenous chickens (Table 1). The SNPs observed in exon 3 of interferon regulatory factor-5 gene are transitions and were present in the three genotypes.

Table 1: Polymorphisms identified in exon 3 of IRF-5 gene in Nigerian indigenous chickens

SNP[a]	Genomic location[b]	Reference allele	Type of mutation	Genotype where SNP occurs[c]	dbSNP ID[d]
33A>G	1: 668,730	A	Transition	NF, NN and FF	rs317511101
48G>A	1: 668,745	G	Transition	NF, NN and FF	rs312902332
57T>C	1: 668,754	T	Transition	NF, NN and FF	rs315149141
174T>C	1: 668,871	T	Transition	NF, NN and FF	rs739389464

[a] Exact position of each SNP on exon 3 based on reference sequence with accession number: NM001031587.1

[b] Location of the SNP on chromosome 1 based on Ensembl Chicken Gallus_gallus 5.0

[c]NF: normal feather chicken, NN: naked neck chicken, FF: frizzle feather chicken

[d]dbSNP ID: identity number of the SNP in single nucleotide polymorphism database

Test of deviation from neutrality

The test of deviation of exon 3 from neutrality is shown in Table 2. Significant ($p<0.05$) Tajima's D value of 2.35 was estimated for exon 3 of IRF-5 gene in naked neck chickens. All the test of neutrality indices obtained for exon 3 of IRF-5 gene in Nigerian indigenous chickens were greater than 1 except Tajima's D value of normal feather chickens (0.93) and Fu's Fs value of naked neck chickens (0.71).

Table 2: Test of deviation of exon 3 from neutrality

Genotype	Tajima's D	Fu's Fs	Fu and Li's D*	Fu and Li's F*
Normal feather	0.93^{NS}	2.48	1.07^{NS}	1.19^{NS}
Naked neck	2.35*	0.71	1.02^{NS}	1.66^{NS}
Frizzle feather	1.59^{NS}	2.65	1.12^{NS}	1.44^{NS}

NS: Not significant, *significant at $p<0.05$.

Selective forces acting on exon 3 of IRF-5 gene in Nigerian indigenous chickens

Selective forces acting on exon 3 of IRF-5 gene in Nigerian indigenous chickens are presented in Table 3. Mean non-synonymous substitutions per non-synonymous site value of 0.00 was estimated for exon 3 of IRF-5 gene in all the three genotypes. Negative selection occurred in exon 3 of IRF-5 gene in the three chicken genotypes.

Table 3: Selective forces acting on exon 3 of IRF-5 gene in Nigerian indigenous chickens

Genotype	dN	dS	dN-dS	Type of selection
Normal feather	0.00	4.00	-4.00	Negative selection
Naked neck	0.00	5.00	-5.00	Negative selection
Frizzle feather	0.00	4.00	-4.00	Negative selection

dN: Mean non-synonymous substitutions per non-synonymous site, dS: Mean synonymous substitutions per synonymous site.

Discussion

The presence of SNPs in exons 3 of IRF-5 gene in Nigerian indigenous chickens was an indication that this region is polymorphic. Equal C↔T and G↔A transition mutations observed in exon 3 of IRF-5 gene in Nigerian indigenous chickens implied that there was no substitution bias in transition mutations in this region. Presence of only transition mutations in exon 3 of IRF-5 gene in Nigerian indigenous chickens was in agreement with the finding of Lesk (2002) who reported that transition mutations are more common than transversions. Comparison of DNA sequences of multicellular animals by Keller et al. (2007) showed an excess of transitional over transversional substitutions and this is due to the relatively high mutation of methylated cytosine to thymine. Transition bias observed in multicellular animals could be caused by a mutational bias due to inherent properties of DNA. Also, in coding regions, the bias could be caused by selection on nonsynonymous transversions. Transition and transversion can change the primary protein structure of the resultant protein although the biochemical difference in the protein product is usually greater for transversion (Zhang, 2000). There is likely to be greater negative selection against transversions and selection will eventually favour DNA repair mechanisms that are efficient in preventing transversions (Keller et al., 2007). Natural selection favours amino acid replacements via transitions and transitions are less severe with respect to the chemical properties of the original and mutant amino acids (Wakeley, 1996).

Positive Tajima's D values observed in exon 3 of IRF-5 gene in Nigerian indigenous chickens was an indication of presence of low levels of low and high frequency mutations which can be linked to balancing selection and decrease in population size (Hahn et al., 2002). Since purifying selection was observed in exon 3, balancing selection can be excluded as the cause of these low levels of low and high frequency mutations and one can safely assume decrease in population size to be the cause.

Antagonistic co-evolution between host and diseases causing organisms is one of the major forces of molecular evolution of organisms (Paterson et al., 2010). Due to this, host species are believed to maintain high standing allelic variability at immune and disease resistance loci, to counter a rapidly evolving and diverse pathogen fauna (Sommer, 2005). Genes implicated in immunity are usually under acute selective pressures in order to resist pathogenic attacks (Downing et al., 2009). Presence of negative selective pressures in exon 3 of IRF-5 gene in Nigerian indigenous chickens reflected the role of the region in elimination of pathogens. This may be attributed to the better survival rate, robustness, tolerance and resistance of Nigerian indigenous chickens to various diseases. Different selective forces act on different immune genes with different functional properties. Afferent (sensing) immune genes involved in pathogen recognition and signalling must recognise many pathogens and thus, positive selection may be observed in them. Efferent immune genes may be more confined and display low tolerance for mutation and recombination, and therefore be maintained by purifying selection. Also, selective patterns can also be shaped by co-evolution between gene and pathogen. For example, TLRs are signalling and receptor molecules that have two major domains which are leucine rich repeats (LRR) and toll interleukin receptor (TIR) domains. LRR domain is responsible for recognition and binding of pathogen ligands and this domain is under balancing selection. TIR domain is complicated in signalling to other components of the immune system cascades and under negative selection (Alcaide and Edwards, 2011). Therefore, selective pressures can vary, even across small portions of the gene, when

the functional properties of genes implicated in immunity differ (Chapman et al., 2016).

Chapman et al. (2016) predicted purifying selection to be the major selective force acting on ancient β -Defensin gene in Waterfowl and they also predicted balancing selection as the major selective force acting on recently duplicated β -Defensin gene in Waterfowl. Downing et al. (2009) also predicted purifying selection to be the major force acting on 26 immune genes out of 64 immune genes they examined in chicken. Presence of only purifying selective force in exon 3 of IRF-5 gene in Nigerian indigenous chickens might also be attributed to years of natural selection and primitive nature of the birds.

Conclusion

Purifying selective events are acting on exon 3 of IRF-5 gene in Nigerian indigenous chickens.

Acknowledgement

The authors are grateful to Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture, Abeokuta for funding this research from grant number, ACE023.

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