# Evolution of Exon 3 Region of Interferon Regulatory Factor-5 Gene in Nigerian Indigenous Chickens

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## ABSTRACT

Evolutionary analysis assists in delineating the origin of organisms and genomic regions. Evolution of interferon regulatory factor-5 gene in Nigerian indigenous chickens was studied in this research. DNA was extracted from 90 Nigerian indigenous chickens and exon 3 of IRF-5 gene was sequenced. The frequency of nucleotides present in exon 3 of IRF-5 gene in Nigerian indigenous chickens was determined using MEGA 6 software while the minimum number of recombination events in the region was determined using DnaSP. MEGA6 software was also used to determine the phylogenetic relationship among exon 3 of IRF-5 in Nigerian indigenous chickens and other chicken genotypes.

Exon 3 of IRF-5 gene in normal feather and naked neck chickens had the same adenine content of 19.46%. Recombination events only took place in naked neck chickens. Phylogenetic analysis revealed that exon 3 of IRF-5 gene in both naked neck and frizzle feather chickens were closely related. We concluded that exon 3 of IRF-5 in Nigerian indigenous chickens contained more purines and the region in naked neck chickens has undergone mutation at most once since the ancestor of the chickens.

(Keywords: chickens, evolution, exon, interferon regulatory factor-5, phylogeny, purines, genetic analysis)

## INTRODUCTION

Chickens are most widely known for their uses as food (meat and eggs), commercial products (feather products and vaccines), and experimental animals in developed countries (Dessie et al., 2011). However, chickens in developing countries have more diverse uses and benefits to households. In the tropics, indigenous chickens are kept for socio-cultural and religious functions such as entertainment, funeral rights, spiritual cleansing, biological clocks, pest control, ingredient of health dishes, gifts and dowry (Njenga, 2005; Dessie et al., 2011).

The Nigerian indigenous chicken is a dual-purpose bird that is raised for meat and egg production in the rural and peri-urban areas of the country (Sonaiya and Olori, 1990). They constitute about 80% of the 120 million birds found in rural areas of Nigeria (Oke, 2011). These native chickens play major roles not only in Nigerian rural economies, but also contribute substantially to the Gross National Product (Momoh et al., 2007). They are kept in small flocks and feed on household refuse, homestead pickings, crop residues, herbage, seeds, grasses, earthworms, insects and small amounts of supplements offered by the flock owners. They contain a highly conserved genetic system with high level of heterozygosity (Wimmers et al., 2000). They are productive and well adapted to the adverse climatic conditions of the tropical environment and low management inputs due to the presence of some major genes (Egahi et al., 2010).

The interferon regulatory factor (IRF) gene family encodes transcription factors with multiple biological functions, which include immune defense against virus, stress response, cell differentiation, reproduction, growth and development (Chen and Royer Jr., 2010). The

IRFs regulate the expression of interferons and interferon-stimulated genes by binding to specific elements in their promoters (Taniguchi et al., 2001).

All IRFs share significant homology in the N-terminal 115 amino acids, which contains the DNA-binding domain and is characterized by five well-conserved tryptophan repeats (Tamura et al., 2008). The DNA-binding domain forms a helix-turn-helix structure and recognizes a DNA sequence known as interferon-stimulated response element (Darnell et al., 1994) which is characterized by the consensus, 5'-AANNGAAA-3' (Fuji et al., 1999). The C-terminal region of IRFs is less well conserved and mediates the interactions of a specific IRF with other family members, transcriptional factors or cofactors, so as to confer specific activities upon each IRF (Meraro et al., 1999). The IRF family consists of nine members in chicken and they include: IRF-1, IRF-2, IRF-4, IRF-5, IRF-6, IRF-7, IRF-8 IRF-9 and IRF-10 (Nehyba et al., 2002).

Interferon regulatory factor-5 is involved in activation of type I interferon genes, inflammatory cytokines and chemokines (Sigurdsson et al., 2005). The IRF-5 gene is also involved in apoptosis and immune response to pathogens (Paun and Pitha, 2007). It is also an important player in macrophage polarization, regulation of B-cell differentiation (Lien et al., 2010) and tumour necrosis factor (Krausgruber, 2011).

Scanty reports are available on chicken IRF-5. So far, no report is available on molecular evolution of the gene in Nigerian indigenous chickens. Keeping this fact in mind, the present study was planned to study the evolution of exon 3 of the gene in order to delineate its origin and ascertain if its alleles are ancient alleles.

## 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$2'E) is in Odeda Local Government Area of Ogun State, Nigeria. The area which lies in the South Western part of Nigeria has a prevailing tropical climate with a mean annual rainfall of about 1037 mm. The mean ambient temperature ranges from 28$^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.

### Source, Sample Size and Management of Experimental Birds

The experimental birds were generated by artificial insemination from mating of indigenous chickens available on the farm. 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. All birds were wing-tagged for proper identification and subjected to the same management practices throughout the experimental period. Commercial feeds were provided for the birds *ad libitum*. 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. Clean water was provided for the birds *ad libitum*. Vaccination schedule for chicken was strictly adhered to and adequate sanitation was practiced to prevent the 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 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 instruction.

### 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 1µl of each gDNA sample on 1.5% agarose gel at 120v for 20 minutes.

## Amplification and Sequencing of Exon 3 of IRF-5 Gene in Nigerian Indigenous Chicken

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 exon 3. For amplification, 1 µl of genomic DNA (~10-15 ng) was added to a reaction mixture containing16.8 µl of nuclease free water, 2.5 µl of 10×PCR buffer, 1.5 µl of 25 mM MgCl2, 1 µl of 5 mM dNTP, 1 µl of 10 UM forward primer, 1 µl of 10 UM reverse primer and 0.2 µl surf Hot Taq.

The PCR conditions included initial denaturation at 96$^{o}$C for 15 minutes, 35 cycles of final denaturation at 95$^{o}$C for 30 seconds, annealing at 62$^{o}$C for 30 seconds, extension at 70$^{o}$C for 1 minute and final extension at 70$^{o}$C 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.

## 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.

## Evolutionary Analysis

The frequency of nucleotides present in exon 3 of IRF-5 gene in Nigerian indigenous chickens was determined using MEGA 6 software.

The minimum number of recombination events (Hudson and Kaplan, 1985) in exon 3 of IRF-5 gene in Nigerian indigenous chickens was determined using DnaSP (Librado and Rozas, 2009).

MEGA6 software was used to determine the phylogenetic relationship among exon 3 of IRF-5 in Nigerian indigenous chickens and other chicken genotypes (Leghorn with accession NM001031587.1 and Red Jungle Fowl with accession number XM015281939.1). The phylogenetic tree was inferred using unweighted pair group method with arithmetic mean based on Jones-Taylor-Thornton matrix-based model. The reliability of the inferred phylogenetic tree was evaluated using bootstrap analysis of 1000 replications.

# RESULTS AND DISCUSSION

## Frequency of Nucleotides and Recombination Events in Exon 3 of IRF-5 Gene in Nigerian Indigenous Chickens

The frequency of nucleotides present in exon 3 of IRF-5 gene in Nigerian indigenous chickens is shown in Table 1. Exon 3 of IRF-5 gene in normal feather and naked neck chickens had the same adenine content of 19.46%. Minimum number of recombination events in exon 3 of IRF-5 gene in Nigerian indigenous chickens is presented in Table 2. Exon 3 of IRF-5 gene in normal feather and frizzle feather chickens had no recombination events whereas their naked neck chicken counterpart had one recombination event between sites 33 and 44.

Historical recombination is important in understanding the role of recombination in the creation of patterns of variability observed in IRF-5 gene in Nigerian indigenous chickens. Recombination is an important evolutionary factor in many organisms. Lower bound (minimum number) were calculated for exon 3 of IRF-5 gene in Nigerian indigenous chickens because many recombination events in the history are typically undetectable, so the actual number of historical events in the gene may be unobtainable (Myers and Griffiths, 2003). Recombination influences genetic diversity in many livestock species.

**Table 1:** Frequency of Nucleotides Present in Exon 3 of IRF-5 Gene in Nigerian Indigenous Chickens.

| Genotype | Purines | | Pyrimidines | | | |
|---|---|---|---|---|---|---|
| | Adenine (%) | Guanine (%) | Cytosine (%) | Thymine (%) | GC (%) | AT (%) |
| NF | 19.46 | 35.97 | 29.67 | 14.90 | 65.64 | 34.36 |
| NN | 19.46 | 35.98 | 29.94 | 14.63 | 65.92 | 34.08 |
| FF | 19.38 | 36.05 | 29.89 | 14.67 | 65.94 | 34.06 |

NF: Normal Feather, NN: Naked Neck, FF: Frizzle Feather
GC: guanine-cytosine content, AT: adenine-thymine content

**Table 2:** Minimum Number of Recombination Events in Exon 3 of IRF-5 Gene in Nigerian Indigenous Chickens.

| Genotype | Minimum number of recombination events |
|---|---|
| Naked Neck | 0 |
| Normal Feather | 1 (33, 48) |
| Frizzle Feather | 0 |

Note: values in parenthesis are sites where recombination events occur.

All genetic variations are ultimately created through mutation, but recombination can create new variants by combining types already present in the population. The presence of recombination event at sites 33 and 48 in exon 3 of IRF-5 gene in naked neck chickens implied that the two loci have mutated at most once since the ancestor of the chickens (Hudson and Kaplan, 1985).

Absence of recombination in exon 3 of IRF-5 gene in normal feather and frizzle feather chickens was an indication that all the variations present in IRF-5 gene in these genotypes were ancient mutations. These ancient mutations might be responsible for functions of the gene which include immune defense against virus, stress response, cell differentiation, reproduction, growth and development (Chen and Royer Jr, 2010). Also, absence of recombination in exon 3 of IRF-5 gene in normal feather and frizzle feather chickens was an indication that each mutation site has the same family tree and that the history of these regions in these chicken genotypes consisted of just one tree.

Recombination generates the history of a sample and the history of a sample is a collection of correlated family tree, one for each site (each nucleotide sequence). The family tree for a site traces the genealogy of a site back to its most common ancestor indicating which sampled variations are most closely related and when the most recent common ancestors occurred (Hudson and Kaplan, 1985).

## Phylogenetic Relationship between Exons 3 of IRF-5 Gene in Nigerian Indigenous Chickens and Other Chicken Genotypes

Phylogenetic relationship between exon 3 of IRF-5 gene in Nigerian indigenous chickens and other chicken genotypes is shown in Figure 1.

Two clades were formed with exon 3 of naked neck and frizzle feather chickens forming sister taxa in one clade while normal feather and red jungle fowl formed another sister taxa in another clade. Phylogenetic analysis revealed that exon 3 of IRF-5 gene in both naked neck and frizzle feather chickens were closely related. Close relationship between exon 3 of naked neck and frizzle feather chickens implied high comparability and evolution from a most common ancestor.

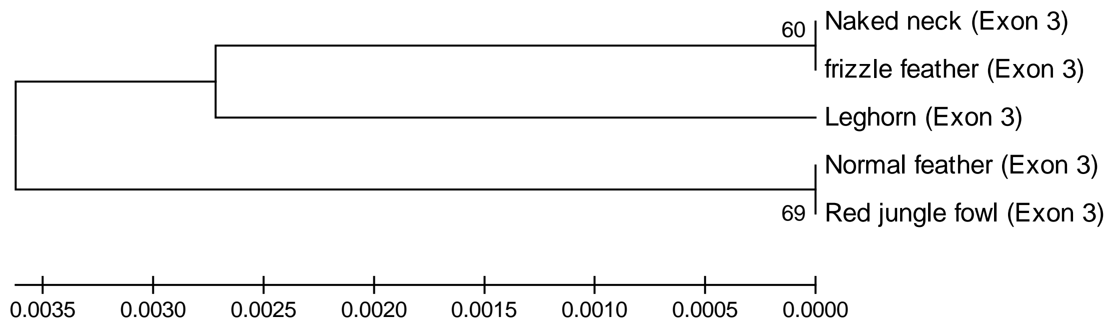


**Figure 1:** Phylogenetic Relationship between Exon 3 of IRF-5 Gene in Nigerian Indigenous Chickens and Other Chicken Genotypes.

## CONCLUSION

Our study on evolution of exon 3 of IRF-5 in Nigerian indigenous chickens revealed that the region contains more purines. Exon 3 of IRF-5 gene in naked neck chickens has undergone mutation at most once since the ancestor of the chickens. Exon 3 of IRF-5 gene of naked neck and frizzle feather chickens were closely related than with other chicken genotypes.

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