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Original Research Article

Identification and characterisation of single nucleotide polymorphisms in interferon regulatory factor-5 gene of Nigerian local chickens

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Abstract

The interferon regulatory factor gene family encodes transcription factors with multiple biological functions, which include reproduction, cell differentiation and immunity. Interferon regulatory factor-5 (IRF-5) gene is involved in immune defence against virus, stress response, activation of type I interferon genes, cell differentiation and growth. This experiment was conducted to identify and characterise single nucleotide polymorphisms in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian local chickens. Exons 3, 4, 5 and 7 of IRF-5 gene were amplified and sequenced. Single nucleotide polymorphisms (SNPs) present in exons 3, 4, 5 and 7 of IRF-5 gene were identified and analysed using Clustal W, DnaSp and SNAP[2] software packages. Four SNPs, rs317511101, rs312902332, rs315149141 and rs739389464, were identified in exon 3 of IRF-5 gene in all the three genotypes. Exon 4 of the gene was conserved while three of the SNPs (rs736423928, 170C>T and rs740736761) identified in exon 7 were shared among the three genotypes. Linkage disequilibrium of 1.00 existed between rs317511101 and rs315149141 polymorphisms identified in exon 3 of normal feathered and frizzle feathered chickens. Mutation rs740736761 identified in exon 7 had the highest polymorphism information content obtainable for any biallelic marker. Most of the SNPs identified in exons 3, 5 and 7 were synonymous and singletons which could not be used for association study. The study concluded that only haplotypes in exons 3 and 7 of IRF-5 gene can be used in marker-assisted selection when improving Nigerian local chickens.

Keywords: Exons; heterozygosity; polymorphism information content; singletons; single nucleotide polymorphisms; synonymous mutations

INTRODUCTION

The Nigerian local chicken is a dual-purpose bird that is raised for meat and egg production (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 (Wong et al., 2017). 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 also have a better flavour of meat and are reared predominantly in the villages because of their inherent advantages over exotic chickens. They are productive and well adapted to the adverse climatic conditions of the tropical environment and low management inputs (Alders et al., 2018). They contain a highly conserved

genetic system with high level of heterozygosity (Wimmers et al., 2000).

The most abundant source of genetic variation is single nucleotide polymorphism, representing a single base change between two individuals at a defined locus (Doveri et al., 2008). Single nucleotide polymorphisms are direct markers as the exact nature of the allelic variants is provided by the sequence information. This sequence variation can have a major impact on how the organism develops and responds to the environment. Single nucleotide polymorphisms markers can be rapidly and cheaply identified through bioinformatics and have many uses in genetics, such as the detection of alleles associated with disease, genome mapping, association studies, genetic diversity, paternity assessment, forensics and inferences of population history (Brumfield et al., 2003; Srinivasan et al., 2016). Single nucleotide polymorphisms within specific genes or genomic regions have also been used to infer phylogenetic relationships between species (Leache and Oaks, 2017).

The interferon regulatory factor (IRF) gene family encodes transcription factors with multiple biological functions, which include immune defence against virus, stress response, cell differentiation, reproduction, growth and development (De et al., 2018). 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 characterised by five well-conserved tryptophan repeats (Tamura et al., 2008). The DNA-binding domain forms a helix-turn-helix structure and recognises a DNA sequence known as interferon-stimulated response elements (ISRE) (Darnell et al., 1994) which is characterised 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 (Esmaeili Reykande et al., 2018). The IRF-5 gene is also involved in apoptosis, immune response to pathogens (Paun and Pitha, 2007), macrophage polarisation, regulation of B-cell differentiation (Lien et al., 2010; Stein et al., 2018) and tumor necrosis factor (Krausgruber, 2011). Chicken IRF-5 gene has been mapped to chromosome 1: 664,415-677,898 in the forward strand (Ensembl Chicken Gallus_gallus 5.0).

Despite the importance of IRF-5 gene and the effects of its polymorphisms in humans and cattle, there is no report of sequence variations of this gene in Nigerian local chickens and their possible usefulness in marker assisted selection. To the best of our knowledge, no report is available on molecular analysis and characterisation of IRF-5 gene in Nigerian local chickens. Keeping this in mind, the present study was planned to identify and characterise polymorphisms in exons 3, 4, 5 and 7 of the gene. We hypothesised that sequence variations identified in these regions are useful for analysis of the evolutionary history, development, assessment of biodiversity and association study in Nigerian local 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°10′N and longitude 3°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 °C in December to 36 °C in February with a yearly average humidity of about 82%. The vegetation represents an interphase between the tropical rainforest and the derived savannah (Durosaro et al., 2019).

Source, sample size and management of experimental birds

The study was carried out for 20 weeks between January and May, 2017. The months of study correspond to late dry and early wet seasons in Nigeria. The experimental birds were generated from mating of parent stocks of local chickens available on the farm through artificial insemination as described by Adeleke et al. (2015). The artificial insemination was done thrice a week (Mondays, Wednesdays and Fridays). The eggs generated were set and hatched at the Programme for Emerging Agricultural Research Leader hatchery located at the University Farm. Ninety birds (27 normal feathered, 45 naked neck and 18 frizzle feathered chickens; 50 males and 40 females) 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. 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 metabolisable energy was fed to the birds from 0 to 8 weeks of age. Grower mash containing 18% crude protein and 10.48 MJ/kg metabolisable energy was fed to the birds from 9 to 12 weeks of age. Clean water was provided for the birds *ad libitum*. Vaccination schedule for chickens was strictly adhered to and adequate sanitation was practised to prevent occurrence of diseases. The protocol for the experiment was approved by Animal Care and Use Committee of College of Animal Science and Livestock Production of the Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. All animal welfare measures were observed during the experiment.

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™ miniprep kit (catalogue number: D3024) following the manufacturer's protocol.

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 IRF-5 in Nigerian local chickens

Polymerase chain reaction (PCR) was carried out using Fwd 5'-TAACCACAACCCAATGATGC-3' and Rev 5'-ATTCCCCCATAAAACACCC-3' primers to amplify 742 bp region covering parts of introns 2–3 and exons 3–5 as well as Fwd 5'-CGGAGCGATATGGAGTG-3' and Rev 5'-TTCTACCTGATGTCCCCC-3' to amplify 747 bp region covering part of introns 6–7, exon 7 and part of introns 7–8. 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 10UM reverse primer and 0.2 µl of 10U/µl surf Hot Taq. The PCR conditions included initial denaturation at 96 °C for 15 minutes, 35 cycles of final denaturation at 95 °C for 30 seconds, annealing at 62 °C (for exons 3–5) and 58 °C (for exon 7) for 30 seconds, extension at 70 °C for 1 minute and final extension at 70 °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 manufacturer's protocol.

Trimming and cleaning of sequences

The nucleotide sequences were trimmed and edited using Bioedit (Hall, 1999) and MEGA 6 (Tamura et al., 2013) software to remove noises in the sequences.

Multiple sequence alignment

The sequences obtained for each exon were aligned with reference exons (NM001031587.1). The alignment was carried out on all the nucleotide sequences using Clustal W software (Thompson et al., 1994) implemented in MEGA 6 software (Tamura et al., 2013).

Identification and analyses of single nucleotide polymorphisms

The SNPs present in each exon of IRF-5 gene in Nigerian local 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).

Allele frequency of each SNP was determined by dividing the frequency of each allele with total sample size for each genotype. Heterozygosity of the SNPs was calculated using the formula proposed by Guo and Elston (1999):

$$\text{Heterozygosity } (H_e) = 1 - (p^2 + q^2)$$

Polymorphism information content (PIC) of the SNPs was calculated using the formula proposed by Botstein et al. (1980):

$$PIC = H_e - 2p^2q^2$$

Where p is the major allele frequency and q is the minor allele frequency.

The linkage disequilibrium among the SNPs was determined by pairwise comparison of r^2 using DnaSP (Librado and Rozas, 2009). DnaSP calculates r^2 as:

$$r^2 = D^2/(P_A(1 - P_A)*P_B(1 - P_B))$$

Where:
r^2 is the linkage disequilibrium
P_A is the frequency of gametes carrying *A* allele.
P_B is the frequency of gametes carrying *B* allele.

$$D \text{ is } P_{AB} - P_A P_B$$

The resultant amino acid variation of each SNP was determined using CodonCode Aligner software (http://www.codoncode.com/aligner). The effect of the amino acid variation on the protein function was predicted using SNAP[2] software (Hecht et al., 2015).

RESULTS

Polymorphisms identified in exons 3, 4, 5 and 7 of interferon regulatory factor-5 gene in Nigerian local chickens

The single nucleotide polymorphisms identified in exons 3, 4, 5 and 7 of interferon regulatory factor-5 gene in Nigerian local chickens are as presented in Table 1. Four single nucleotide polymorphisms, 33A>G, 48G>A, 57T>C and 174T>C, were identified in exon 3 of interferon regulatory factor-5 gene in Nigerian local chickens. The SNPs observed in exon 3 of interferon regulatory factor-5 gene are transitions and were present in normal feathered, naked neck and frizzle feathered chickens. The four transitions (33A>G, 48G>A, 57T>C and 174T>C) identified in exon 3 of interferon regulatory factor-5 gene in Nigerian local chickens have been previously reported in database of single nucleotide polymorphism (dbSNP) and have identifiers: rs317511101, rs312902332, rs315149141 and rs739389464, respectively.

Single nucleotide polymorphisms were not identified in exon 4 of IRF-5 gene in Nigerian local chickens. However, six polymorphisms (two transitions and four transversions) were identified in exon 5 of IRF-5 gene in normal feathered chickens. The SNPs present in exon 5 of IRF-5 gene in normal feathered chickens have not been previously reported in dbSNP. Polymorphisms were, however, not identified in exon 5 of IRF-5 gene of naked neck and frizzle feathered chickens.

Five SNPs with genomic locations 1:674974, 1:675081, 1:675132, 1:675138 and 1:675315 were identified in exon 7 of IRF-5 gene in Nigerian local chickens. The five SNPs included: 6G>A, 113G>C, 164T>C (rs736423928), 170C>T and 347G>A (rs740736761). Polymorphisms, 6G>A and 113G>C, were specific to exon 7 of IRF-5 gene in naked neck chickens while the other three polymorphisms were present in the three genotypes.

Overall, nearly 67% of the SNPs observed in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian local chickens were transitions.

Table 1. Polymorphisms identified in exons 3, 4, 5 and 7 of interferon regulatory factor-5 gene in Nigerian indigenous chickens

Region	SNP[a]	Genomic location[b]	Type of mutation	Genotype where SNP occurs	dbSNP ID
Exon 3	33A>G	1: 668,730	Transition	NF, NN and FF	rs317511101
	48G>A	1: 668,745	Transition	NF, NN and FF	rs312902332
	57T>C	1: 668,754	Transition	NF, NN and FF	rs315149141
	174T>C	1: 668,871	Transition	NF, NN and FF	rs739389464
Exon 4	Not present				
Exon 5	6G>T	1: 669,257	Transversion	NF	FKR
	14T>A	1: 669,265	Transversion	NF	FKR
	17C>T	1: 669,268	Transition	NF	FKR
	19C>T	1: 669,270	Transition	NF	FKR
	20T>A	1: 669,271	Transversion	NF	FKR
	23G>C	1: 669,274	Transversion	NF	FKR
Exon 7	6G>A	1: 674,974	Transition	NN	FKR
	113G>C	1: 675,081	Transversion	NN	FKR
	164T>C	1: 675,132	Transition	NF, NN and FF	rs736423928
	170C>T	1: 675,138	Transition	NF, NN and FF	FKR
	347G>A	1: 675,315	Transition	NF, NN and FF	rs740736761

[a] Exact position of each SNP on each exon based on Ensembl Chicken Gallus_gallus 5.0 with transcript identity: ENSGALT00000068201.1
[b] Location of the SNP on chromosome 1 based on Ensembl Chicken Gallus_gallus 5.0
dbSNP ID: identity number of the SNP in single nucleotide polymorphism database
NF: normal feathered chicken, NN: naked neck chicken, FF: frizzle feathered chicken FKR: First known report

Table 2. Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 3 of IRF-5 gene in Nigerian indigenous chickens

Genotype	SNP	Major allele frequency	Heterozygosity	Polymorphism information content
Normal feathered	rs317511101	0.74	0.38	0.31
	rs312902332	0.93	0.14	0.13
	rs315149141	0.74	0.38	0.31
	rs739389464	0.67	0.44	0.35
Naked neck	rs317511101	0.53	0.50	0.37
	rs312902332	0.73	0.39	0.32
	rs315149141	0.60	0.48	0.37
	rs739389464	0.69	0.43	0.34
Frizzle feathered	rs317511101	0.56	0.49	0.37
	rs312902332	0.89	0.20	0.18
	rs315149141	0.56	0.49	0.37
	rs739389464	0.56	0.49	0.37

Major allele frequency, heterozygosity and polymorphic information content of SNPs identified in exon 3 of IRF-5 gene in Nigerian local chickens

The major allele frequency, heterozygosity and polymorphic information content of SNPs identified in exon 3 of IRF-5 gene in Nigerian local chickens are presented in Table 2. The major allele frequency of polymorphisms identified in exon 3 of IRF-5 gene in normal feathered chickens ranged from 0.67 to 0.93 with rs739389464 having the lowest value. The heterozygosity of the SNPs observed in exon 3 of IRF-5 gene in normal feathered chickens ranged from 0.14 to 0.44. A heterozygosity value of 0.38 was observed in rs317511101 and rs315149141. A polymorphism information content of 0.31 was observed for rs317511101 and rs315149141 in exon 3 of IRF-5 gene in normal feathered chicken.

The major allele frequency of polymorphisms identified in exon 3 of IRF-5 gene in naked neck chickens ranged from 0.53 to 0.73 with rs317511101 having the least major allele frequency. The heterozygosity of SNPs present in exon 3 of IRF-5 gene in naked neck chickens ranged from 0.39 to 0.50 with rs317511101 having the highest heterozygosity value.

A polymorphism information content of 0.37 was observed for rs317511101 and rs315149141 in exon 3 of IRF-5 gene in naked neck chickens.

Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 5 of IRF-5 gene in Nigerian local chickens

All the SNPs identified in exon 5 of IRF-5 gene in normal feathered chickens have a major allele frequency of 0.96 (Table 3). The SNPs in exon 5 of IRF-5 gene in normal feathered chickens have a heterozygosity value of 0.07 and PIC value of 0.07.

Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 7 of IRF-5 gene in Nigerian local chickens

The major allele frequency of SNPs observed in exon 7 of IRF-5 gene in normal feathered chickens ranged from 0.52 to 0.64 with SNP 170C>T having the highest major allele frequency (Table 4). The PIC of SNPs observed

Table 3. Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 5 of IRF-5 gene in Nigerian indigenous chickens

Genotype	SNP	Major allele frequency	Heterozygosity	Polymorphism information content
Normal feathered	6G>T	0.96	0.07	0.07
	14T>A	0.96	0.07	0.07
	17C>T	0.96	0.07	0.07
	19C>T	0.96	0.07	0.07
	20T>A	0.96	0.07	0.07
	23G>C	0.96	0.07	0.07
Naked neck	Not present			
Frizzle feathered	Not present			

Table 4. Major allele frequency, heterozygosity and polymorphism information content of SNPs identified in exon 7 of IRF-5 gene in Nigerian indigenous chickens

Genotype	SNP	Major allele frequency	Heterozygosity	Polymorphism information content
Normal feathered	rs736423928	0.55	0.50	0.37
	170C>T	0.64	0.46	0.36
	rs740736761	0.52	0.50	0.38
Naked neck	6G>A	0.97	0.06	0.06
	113G>C	0.97	0.06	0.06
	rs736423928	0.61	0.48	0.36
	170C>T	0.77	0.35	0.29
	rs740736761	0.74	0.38	0.31
Frizzle feathered	rs736423928	0.74	0.39	0.31
	170C>T	0.83	0.29	0.25
	rs740736761	0.74	0.39	0.31

in exon 7 of IRF-5 gene in normal feathered chicken ranged from 0.36 to 0.38 with mutation 170C>T having the lowest value. Major allele frequency of 0.97 was observed in both 6G>A and 113G>C polymorphisms observed in exon 7 of IRF-5 gene in naked neck chickens. Heterozygosity value of 0.06 was observed for both mutations 6G>A and 113G>C in exon 7. The highest heterozygosity value of 0.48 was observed for rs736423928 in exon 7 of naked neck chickens. The PIC value of SNPs present in exon 7 of IRF-5 gene in naked neck chickens ranged from 0.06 to 0.31. Also, mutations 6G>A and 113G>C in exon 7 of IRF-5 gene in naked neck chickens had a PIC value of 0.06. A major allele frequency of 0.74 was also observed in rs736423928 and rs740736761 out of the three SNPs identified in exon 7 of IRF-5 gene in frizzle feathered chickens. The PIC of SNPs observed in exon 7 of IRF-5 gene in frizzle feathered chickens ranged from 0.25 to 0.31 with mutation 170C>T having the least value.

Amino acid variations and predicted effects of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian local chickens

The resultant amino acid variations and predicted effects of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian local chickens is presented in Table 5. All the SNPS identified in exon 3 of IRF-5 gene in

Table 5. Resultant amino acid variations and predicted effects of SNPs identified in exons 3, 4, 5 and 7 of IRF-5 gene in Nigerian indigenous chickens

Region	SNP	Codon change	Amino acid variation	Type of mutation	Predicted effect	Score	Accuracy (%)
Exon 3	33A>G	GC**A**→GC**G**	Alanine→Alanine	Synonymous	Neutral	–99	97
	48G>A	CC**G**→CC**A**	Proline→Proline	Synonymous	Neutral	–99	97
	57T>C	GC**T**→GC**C**	Alanine→Alanine	Synonymous	Neutral	–99	97
	174T>C	GC**T**→GC**C**	Alanine→Alanine	Synonymous	Neutral	–99	97
Exon 4	Not present						
Exon 5	6G>T	CA**G**→CA**T**	Glutamine→Histidine	Nonsynonymous	Neutral	–75	87
	14T>A	A**T**G→A**A**G	Methionine→Lysine	Nonsynonymous	Effect	5	53
	17C>T	T**C**T→T**T**T	Serine→Phenylalanine	Nonsynonymous	Neutral	–27	61
	19C>T	**C**TG→**T**TG	Leucine→Leucine	Synonymous	Neutral	–99	97
	20T>A	C**T**G→C**A**G	Leucine→Glutamine	Nonsynonymous	Neutral	–45	72
	23G>C	A**G**T→A**C**T	Serine→Threonine	Nonsynonymous	Neutral	–59	78
Exon 7	6G>A	**G**AC→**A**AC	Aspartic acid→Asparagine	Nonsynonymous	Effect	40	71
	113G>C	CC**G**→CC**C**	Proline→Proline	Synonymous	Neutral	–99	97
	164T>C	CC**T**→CC**C**	Proline→Proline	Synonymous	Neutral	–99	97
	170C>T	CC**C**→CC**T**	Proline→Proline	Synonymous	Neutral	–99	97
	347G>A	CG**G**→CG**A**	Arginine→Arginine	Synonymous	Neutral	–99	97

Positions where the SNPs occurred are in bold.

Table 6. Linkage disequilibrium (based on r^2 statistics) among SNPs identified in exon 3 of IRF-5 gene in Nigerian indigenous chickens

Genotype	SNP 1	SNP 2			
		rs317511101	**rs312902332**	**rs315149141**	**rs739389464**
Normal feathered	rs317511101		0.028^{NS}	1.000***	0.701***
	rs312902332			0.028^{NS}	0.160^{NS}
	rs315149141				0.701***
	rs739389464				
		rs317511101	**rs312902332**	**rs315149141**	**rs739389464**
Naked neck	rs317511101		0.214**	0.762***	0.283***
	rs312902332			0.242**	0.164**
	rs315149141				0.301***
	rs739389464				
		rs317511101	**rs312902332**	**rs315149141**	**rs739389464**
Frizzle feathered	rs317511101		0.100^{NS}	1.000***	0.640**
	rs312902332			0.100^{NS}	0.100^{NS}
	rs315149141				0.640**
	rs739389464				

** significant at $p < 0.01$, *** significant at $p < 0.001$, ^{NS} not significant

Nigerian local chickens were synonymous SNPs and were predicted to have neutral effect on the resultant protein function with accuracy of 97%.

All the SNPs identified in exon 5 of IRF-5 gene in normal feathered chickens were predicted to have neutral effect on the resultant protein function except mutation 14T>A. Also, mutation 6G>A identified in exon 7 of IRF-5 gene in naked neck chickens was predicted to have effect on the resultant protein function with accuracy of 71%.

Linkage disequilibrium among SNPs identified in exon 3 of IRF-5 gene in Nigerian local chickens

Linkage disequilibrium among SNPs identified in exon 3 of IRF-5 gene in Nigerian local chickens is shown in Table 6. Linkage disequilibrium among the SNPs present in exon 3 of IRF-5 gene in normal feathered chickens ranged from 0.028 to 1.000. A very low LD of 0.028 was observed between rs317511101 and rs312902332 as well as between rs312902332 and rs315149141. A very high LD of 1.000 was observed between rs317511101 and rs315149141 in normal feathered chickens. The linkage disequilibrium among SNPs identified in exon 3 of IRF-5 gene in naked neck chickens ranged from 0.164 to 0.762 with the highest value observed between rs317511101 and rs315149141. A high significant ($p<0.001$) LD of 0.640 was observed between rs317511101 and rs739389464 in exon 3 of IRF-5 gene in frizzle feathered chickens.

Linkage disequilibrium among SNPs identified in exon 5 of IRF-5 gene in Nigerian local chickens

Linkage disequilibrium among SNPs identified in exon 5 of IRF-5 gene in Nigerian local chickens is presented in Table 7. Very high linkage disequilibrium of 1.00 was observed among all the SNPs identified in exon 5 of IRF-5 gene in normal feathered chickens. There were no pairwise comparisons for naked neck and

Table 7. Linkage disequilibrium (based on r^2 statistics) among SNPs identified in exon 5 of IRF-5 gene in Nigerian indigenous chickens

Genotype	SNP 1	SNP 2					
		6G>T	**14T>A**	**17C>T**	**19C>T**	**20T>A**	**23G>C**
Normal feathered	6G>T		1.00*	1.00*	1.00*	1.00*	1.00*
	14T>A			1.00*	1.00*	1.00*	1.00*
	17C>T				1.00*	1.00*	1.00*
	19C>T					1.00*	1.00*
	20T>A						1.00*
	23G>C						
Naked neck	No pairwise comparison						
Frizzle feathered	No pairwise comparison						

* significant at $p < 0.05$

Table 8. Linkage disequilibrium (based on r^2 statistics) among SNPs identified in exon 7 of IRF-5 gene in Nigerian indigenous chickens

Genotype	SNP 1	SNP 2				
				rs736423928	170C>T	rs740736761
Normal feathered	rs736423928				0.202*	0.270**
	170C>T					0.369***
	rs740736761					
		6G>A	113G>C	rs736423928	170C>T	rs740736761
Naked neck	6G>A		0.001^{NS}	0.053^{NS}	0.010^{NS}	0.012^{NS}
	113G>C			0.021^{NS}	0.010^{NS}	0.012^{NS}
	rs736423928				0.184*	0.220*
	170C>T					0.546***
	rs740736761					
				rs736423928	170C>T	rs740736761
Frizzle feathered	rs736423928				0.596**	0.601**
	170C>T					0.596***
	rs740736761					

* significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$, ^{NS} not significant

frizzle feathered chickens as they contained no single nucleotide polymorphisms.

Linkage disequilibrium among SNPs identified in exon 7 of IRF-5 gene in Nigerian local chickens

Linkage disequilibrium among SNPs identified in exon 7 of IRF-5 gene in Nigerian local chickens is shown in Table 8. A highly significant ($p < 0.001$) moderate LD of 0.369 was observed between 170C>T and rs740736761 in exon 7 of IRF-5 gene in normal feathered chickens.

Linkage disequilibrium among SNPs present in exon 7 of IRF-5 gene in naked neck chickens varied from 0.001 to 0.546. A very low LD of 0.001 was observed between 6G>A and 113G>C while LD of 0.546 was observed between 170C>T and rs740736761. Linkage disequilibrium of 0.596 was observed between rs736423928 and 170C>T as well as 170C>T and rs740736761 in exon 7 of IRF-5 gene in frizzle feathered chickens.

DISCUSSION

The presence of SNPs in exons 3 and 7 of IRF-5 gene in Nigerian local chickens is an indication that these regions are polymorphic. Absence of SNPs in exon 4 of IRF-5 gene in Nigerian local chickens and exon 5 of naked neck and frizzle feathered chickens is an indication that these regions are totally conserved. Conserved genomic regions are likely to be responsible for gene functions as sequence conservation points to an important biological role (Skittrall et al., 2019). Skittrall et al. (2019) also reported that proteins that are coded for by conserved sequences provide some vital functions for the organism. The conserved amino acid sequence of exon 4 of IRF-5 gene of Nigerian local chickens as well as exon 5 of naked neck and frizzle feathered chickens will probably correspond to the active site of their proteins (Lesk, 2002). Several other genomic regions that are related to immunity have been found to be conserved in different livestock species. Cluster of differentiations 4 and 8 have been found to be conserved and they play an active role in T-cell recognition and activation by binding to their respective class I and II major histocompatibility ligands on their antigen presenting cells acting as co-receptor for polymorphic T-cell receptor (Miceli et al., 1990).

Equal C↔T and G↔A transition mutations observed in exon 3 of IRF-5 gene in Nigerian local chickens implied that there was no substitution bias in transition mutations in this region. Presence of 67% transitions in exons 3, 5 and 7 of IRF-5 gene in Nigerian local chickens is in agreement with the finding of Payne et al. (2019) who reported that transition mutations are more common than transversions. Comparison of DNA sequences of metazoan 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 metazoans could be caused by a mutational bias due to intrinsic properties of DNA. Also, in coding regions, this bias could be explained by selection on nonsynonymous transversions. Transition and transversion can change the amino acid composition of the resultant protein but the biochemical difference in the protein product tends to be greater for transversion (Lyons and Lauring, 2017). There is likely to be a greater purifying selection against transversions and selection

could therefore 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). Presence of more transversions in exon 5 of IRF-5 gene in normal feathered chickens is in agreement with the findings of Hale et al. (2009) who reported higher transversion sites in the regulatory genes such as Endonuclease reverse transcriptase and TC1-like transposase. Exon 5 of IRF-5 gene in normal feathered chickens might be responsible for encoding transcription factors with multiple biological functions observed in the gene.

Diversity at immune gene loci has been linked to fitness (Brambilla et al., 2017). Heterozygosity quantifies within individual genetic diversity and is also related to inbreeding. Low heterozygosity of SNPs observed in exon 5 of IRF-5 gene in normal feathered chickens and exon 7 of IRF-5 gene in naked neck chickens may have deleterious effects on their fitness (Wright, 1992). Low heterozygosity can affect fitness in natural populations (Bateson et al., 2016) as reduced genetic diversity may interact with extrinsic stressors, such as disease, to influence population dynamics (Forcada and Hoffman, 2014). There is also a positive correlation between heterozygosity and immunity (Brock et al., 2013). Heterozygosity-fitness correlation (HFC) have been studied in many populations and the explanation of HFC to inbreeding depression is that of linkage between one or few neutral markers and functional genes under balancing selection which could give rise to the frequently observed pattern of heterosis (Balloux et al., 2004). High heterozygosity is generally beneficial for individuals because it decreases expression of recessive deleterious alleles (Keller and Waller, 2002). A very high heterozygosity of 0.5 observed in polymorphisms rs317511101 in exon 3 of naked neck chickens as well as rs736423928 and rs740736761 in exon 7 of normal feathered chickens indicates that recessive deleterious alleles in these exons may not be expressed. HFC arises by genome wide effects of heterozygosity, which is usually called general effect hypothesis (Hansson and Westerberg, 2002). However, it is strongly debated if heterozygosity measured across a set of genetic markers could reflect genome-wide heterozygosity and also inbreeding level. Alternatively, marker heterozygosity might reflect heterozygosity states at closely linked loci only (Balloux et al., 2004). Hence, the local effect hypothesis that HFC occurs due to LD between genetic markers and loci under selection.

Single nucleotide polymorphisms which are biallelic markers have a maximum PIC of 0.38 (Chen et al., 2017) and this was obtained for mutation rs740736761 in exon 7 of normal feathered chickens. The implication of this high PIC value is that the marker is highly informative for linkage studies and any normal feathered chicken chosen at random at this locus is likely to be heterozygous for that marker. The higher PIC of SNPs generally observed in exon 7 of normal feathered chickens is an indication of genetic variation and a selective potentially using this exon for marker assisted selection in this genotype (Platten et al., 2019). Various PICs values observed for different exons and different genotypes may be influenced by many factors. Factors such as breeding behaviour of species, genetic diversity, sensitivity of genotyping method and genomic location of the markers affect PIC of SNPs (Singh et al., 2013).

All the SNPs identified in exon 3 of IRF-5 gene in Nigerian local chickens were synonymous mutations which are not expected to cause any amino acid variation. Although these SNPs are not expected to have effect on protein function, Bin et al. (2019) reported that synonymous mutations have effect on disease. Silent mutations are now widely acknowledged to be able to cause changes in protein expression, conformation and function. There is also codon usage bias vis-à-vis synonymous codon, suggesting that synonymous codons are under evolutionary pressure (Chamary et al., 2006). Synonymous mutations can also result in aberrant mRNA splicing that can cause disease (Cartegni et al., 2002). Evidence by Nackley (2006) also suggested that synonymous SNPs could affect mRNA stability and thus protein expression and enzymatic activity. Diseases like pulmonary sarcoidosis, haemophilia, cervical cancer, vulva cancer and non-small-cell lung carcinoma in humans have been associated with synonymous mutations through codon usage bias (Sauna and Kimchi-Sarfaty, 2011). Chen et al. (2010) conducted a survey of some associations between diseases and SNPs curated from 2,113 reports studying genetic association and they concluded that non-synonymous and synonymous SNPs shared similar likelihood and effect size for disease association. There is also a range of mechanisms by which synonymous mutations can affect the yields of active, correctly folded protein and thus have an impact on physiological activity (Chamary et al., 2006).

Linkage disequilibrium is a property of SNPs on a contiguous stretch of genomic sequence that describes the degree to which an allele of one SNP is inherited or correlated with an allele of another SNP

within a population (Bush and Moore, 2012). Linkage disequilibrium of 0.001 between 6G>A and 113G>C in exon 7 of IRF-5 gene in naked neck chickens implied that there was almost linkage equilibrium between the two SNPs and there was almost non-joint evolution of the two SNPs. Linkage disequilibrium between two SNPs reflects the history of natural selection, gene conversion and other forces that cause gene-frequency evolution (Slatkin, 2008). The very low LD between 6G>A and 113G>C in exon 7 of IRF-5 gene in naked neck chickens reflects statistical independence and random association. Genetic drift balanced by mutation and/or recombination could also have caused this very low LD as reported by Ohta and Kimura (1969).

LD of 1 obtained for rs317511101 and rs315149141 in exon 3 of IRF-5 gene in normal feathered and frizzle feathered chickens as well as among all the SNPs in exon 5 of normal feathered chickens indicates that all these SNPs can affect the same immune traits. These SNPs convey similar information and can be used as tag SNPs because their alleles tag the surrounding stretch of LD (Bush and Moore, 2012). The response to selection of any one of these SNPs might be accelerated or impeded by selection affecting the other. Selection alone can increase LD and this occurs when fitness are more than multiplicative, meaning that the average fitness of an individual carrying *AB* haplotype exceeds the product of the average fitness of individuals carry *A* or *B* alone (Felsentein, 1965). Also, LD of 1 observed in exon 5 of IRF-5 gene in normal feathered chickens might have resulted from very high proximity of the SNPs. Closely linked polymorphic SNPs tend to be in strong LD with one another (International HapMap Consortium, 2007). High level of LD among SNPs is also assumed to be true for alleles that are involved in immunity (Slatkin, 2008). LD of 1 in exon 5 of IRF-5 gene in normal feathered chickens might have also resulted from strong positive selection occurring in this region as positive selection quickly increases the frequency of advantageous alleles, with the result that linked loci remain unusually in strong LD with that allele which results in genetic hitch-hiking (Maynard and Haigh, 2007). LD of 1 observed in exon 5 of IRF-5 gene in normal feathered chickens might also imply that the alleles of SNPs observed in this region are young or that much time has not passed since the allele arouse by mutation (Slatkin, 2008). Formation of new allele by immune genes such as IRF-5 might have resulted from host-parasite/pathogen coevolution (Croze et al., 2016).

Presence of six singleton variable sites out of 31 base pairs in exon 5 of IRF-5 gene in normal feathered chickens was an indication of pathogen/transcription signal pressure effect on the region. Rare allele variants may rapidly increase in frequency when pathogen pressure is strong and resistance enhances animal fitness but later may become selectively neutral due to pathogen counter-adaptation or a low frequency of attack called novel allele advantage (Lazzaro and Clark, 2003).

CONCLUSION AND RECOMMENDATION

All the SNPs identified exons 3, 5 and 7 of IRF-5 gene in Nigerian local chickens are either synonymous or singletons and cannot be used for association study. Only the haplotypes in exons 3 and 7 of IRF-5 gene can be used in marker-assisted selection when improving Nigerian local chickens.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest with respect to research, authorship and publication of this article.

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