Genetic Diversity of *zyxin* and *TNFRSF1A* genes in Nigerian Local Chickens and Nera Black Chickens

Adeyemi Sunday ADENAIKE[1] (✉)
Sunday Olusola PETERS[2]
Adeboye Olusesan FAFIOLU[3]
Matthew Adeleke ADELEKE[4]
Micheal Irewole TAKEET[5]
Mathew WHETO[1]
Olufunmilayo Ayoka ADEBAMBO[1]
Christian Obiora N. IKEOBI[1]

Summary

This study was conducted to assess the population and genetic diversity of *TNFRSF1A* and *zyxin* genes among the Nigerian indigenous (Naked neck, Normal and Frizzle feather) and Nera Black chickens (exotic chicken). Blood samples were collected from 400 chickens belonging to the genotypes described above and genomic DNA extracted using commercially available kit. Genomic regions containing *zyxin* and *TNFRSF1A* genes were amplified, sequenced and analyzed using Bioedit, DnaSP, MEGA and PANTHER software. The results from analysis revealed that Naked neck and Normal feather chickens had the highest mean value of nucleotide substitutions per sites (D_{xy}) (0.081), while Frizzle and Nera Black local chickens had the lowest D_{xy} value of 0.065 for *TNFRSF1A* gene sequences. The results from the analysis of *zyxin* gene sequence revealed that Frizzle and Normal feather chickens had the highest D_{xy} (0.6551), while Normal feather and Naked neck chickens had the lowest D_{xy} (0.0739). Nera Black chickens *TNFRSF1A* gene sequence had the highest mean values of haplotype diversity and average number of nucleotide difference (0.923 and 3.967 respectively). In *zyxin* sequences, the highest average number of nucleotide difference (3.143) and nucleotide diversity (0.00489) were detected in the Frizzle feather chicken. The analyses of these two genes sequences revealed high nucleotide divergence and haplotypes diversity. The results of divergence, haplotypes diversity and phylogenetic tree indicated a historically restricted gene flow among the genotypes.

Key words

Genetic diversity, *TNFSFR1A* gene, *zyxin* gene, Indigenous chickens

[1] Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta, Nigeria. P.M.B. 2240 Abeokuta, Nigeria

[2] Department of Animal Science, Berry College, Mount Berry, GA, 30149, USA

[3] Department of Animal Nutrition, Federal University of Agriculture, Abeokuta, Nigeria

[4] Discipline of Genetics, School of Life Sciences, University of KwaZulu-Natal (Westville Campus), P/Bag X54001 Durban 4000, South Africa

[5] Department of Veterinary Microbiology and Parasitology, Federal university of Agriculture, Abeokuta, Nigeria

✉ Corresponding author: adenaikeas@funaab.edu.ng

Received: May 25, 2018 | Accepted: October 17, 2018

Introduction

The indigenous chicken in Nigerian have developed over the years certain valuable genetic traits such as their capacity to survive on low quality feed, climatic stress and tolerance to infectious diseases (Adenaike et al., 2018). Chickens are important pools of useful genes and genetic improvement of indigenous chicken is crucial. Many breeds of livestock in third world countries may lose their germplasm due to extensive crossbreeding as well as uncontrollable mating in extensive management systems (Groeneveld et al., 2010).

Nigerian chickens have been classified into three major genes (Naked neck, Normal and Frizzle feather) based on the presence or absence of the feather distribution and feather structure genes with reported implications on productive and adaptative traits (Fassil et al., 2010; Adeleke et al., 2015). To obtain better understanding of genetic variation among indigenous chickens and formulate conservation policies, better knowledge of their genetic annotation and diversity should be pursued. This would enable breeders and researchers to resourcefully strike a balance between genetic improvement and conservation of native germplasm of our indigenous chicken.

TNFRSF1A is a multifunctional pro-inflammatory cytokine that belongs to the tumour necrosis factor superfamily and has beneficial properties (Um et al., 2005). *TNFRSF1A* encodes 55kDa TNF-α receptor. TNF-α binds to *TNFRSF1A* as a key mediator in the inflammatory response with pleiotropic activities including increased expression of adhesion molecules, induction of cytokine secretion, activation of leukocytes as well as host defense against intracellular pathogens (Rezaei, 2006). *Zyxin* encodes a protein that functions as a component of focal adhesion complexes, tumour metastasis and wound healing (Moon et al., 2006).

The activities of these two genes were further corroborated by Hong et al. (2009) who reported their immunological function and involvement in protective immunity to coccidiosis in chicken. Based on available information on these genes, studying their variation in Nigerian indigenous chicken will provide useful information for selective breeding programs and conservation planning, which allows chicken populations to adapt as environmental conditions change. We sequenced *zyxin* and *TNFRSF1A* loci from Nigerian indigenous and Nera Black (an exotic but locally adapted to Nigeria) chickens in order to produce genomic resources for understanding evolution of the genes and determine the extent of genetic diversity between and within the chicken populations.

Materials and Methods

Experimental Animals

Nigerian local chickens comprising Naked neck, Frizzle and Normal feather were sourced (based on feather structure and distribution) from different villages in the southwest of Nigeria to form the foundation stock which was maintained at the University Farms. The same genotypes were mated using artificial insemination to generate 100 progenies each from the pure mating of the Naked neck, Normal and Frizzle feather. A total of 100 chicks of Nera Black chicken were purchased and raised with them for this study at the Poultry Breeding Unit of the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta, Nigeria.

Sample Collection and DNA Isolation

2.5ml of whole blood was collected through the wing web from a total of 400 Nigerian local and Nera Black chickens. Genomic DNA was extracted from the bloods using the Qiagen DNA easy© blood kit-Animal blood following manufacturer's Protocol. The concentration and purity of the DNA extracted was determined using a Nanodrop.

PCR Amplification and DNA Sequencing

Polymerase chain reaction (PCR) was performed to amplify a 608-bp fragment of *TNFRSF1A* gene using forward (5′-CAGAGATTCAGAAGGGGTTGC-3′) and reverse (5′-TAATTGCTTTTTGCTACTTCTGCT-3′) primers. A 561-bp fragment of *zyxin* gene was amplified with forward (5′-ACCCCAGGGACCCGTATGAC-3′) and reverse (5′-GGGTCCTTGCGCTGCTGTG-3′) primers. Amplifications for the *TNFRSF1A* and *zyxin* primer sets followed Hong et al. (2009) using annealing temperatures of 55 and 58 respectively. The PCR products were sequenced directly and unidirectionally using forward primers that were used to amplify the amplicons with an ABI 3730 DNA analyzer (Applied Biosystem, Foster City, CA) in Cornell Core Laboratory, Cornell University, Ithaca, New York USA.

Data Analysis

After sequencing, sequences that have poor quality were excluded from the analyses. CodonCode Aligner was used to trim the sequences and remove contaminants (low complexity regions can provide an artifactual basis for cluster membership) from each of the sequences. *Zyxin* and *TNFRSF1A* nucleotide sequences were aligned and analyzed in Bioedit program (version 5.0.9; Hall, 1999) and translated into the corresponding amino acid sequences using the NCBI ORF Finder along with MEGA software package (Tamura et al., 2013). The DNA sequences of the *zyxin* and *TNFRSF1A* were aligned using the Clustal-X multiple sequence alignment program (Thompson et al., 1994). The MEGA 6.1 program (Tamura et al., 2013) was used to estimate relative rates of non-synonymous (dN) and synonymous substitutions (dS) according to Nei and Gojobori (1986), using Jukes and Cantor's (1969) correction for multiple hits. To test the type of selection in operation at the locus of each gene, the relative abundance of synonymous and non-synonymous substitutions using a Z-test (Nei and Kumar, 2000) was compared. The variance of dN and dS was estimated using the bootstrap method implemented in MEGA, with 1000 replications (Schneider et al., 2000).

In silico functional analysis of non-synonymous mutations was obtained using Panther (Thomas et al., 2003), a program that estimates the likelihood that a particular non-synonymous (amino acid change) coding SNP will cause a functional impact on the protein. It calculates the substitution Position-Specific Evolutionary Conservation (subPSEC) score based on an alignment of evolutionarily-related proteins. The probability that a given variant will cause a deleterious effect on protein function

is estimated by $P_{deleterious}$, such that a subPSEC score of -3 corresponds to a $P_{deleterious}$ of 0.5 (Brunham et al., 2005). The subPSEC score is the negative logarithm of the probability ratio of the wild-type and mutant amino acids at a particular position. Panther subPSEC scores derived from the probabilities of observing the variant amino acids in a Panther Hidden Markov Model are continuous values from 0 to -10. When subPSEC =0, the substitution is interpreted as functionally-neutral, whereas more negative values of subPSEC predict more deleterious substitutions (Brunham et al., 2005).

MEGA was also used to perform phylogenetic analyses of the genes separately. Two independent phylogenetic methods (neighbour-joining and maximum parsimony) were used to confirm the reliability of the observed phylogenetic patterns. The neighbour-joining tree was constructed using Jukes and Cantor (1969) genetic distances. Bootstrap re-sampling of 1000 multiple data sets provided estimates of relative confidence for nodes in the phylogenetic trees. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated.

Results

The *TNFRSF1A* sequences with accession numbers KY494915 - KY494922 and *zyxin* sequences with accession numbers KY560190 - KY560196 have been deposited in GenBank.

Polymorphism and Genetic Diversity within *TNFRSF1A* gene of Four Chicken Genotypes

The results of DnaSP analyses indicated that the selected region (1- 920bp) of the 132 sequences from the four genotypes (Normal feather = 34, Naked neck = 40, Frizzle feather = 26 and Nera Black = 32) had 531 nucleotide sites excluding sites with gaps (389). There were non-variable and variable sites that included 56 single variable sites and parsimony informative sites. The haplotype diversity (0.954) and average number of nucleotide diversity (k=36.667) for all chicken *TNFRSF1A* sequences were higher than the highest values in Nera Black (H_d=0.923, k=3.967). The genetic diversity of *TNFRSF1A* gene in four chicken genotypes is shown in Table 1. The number of haplotypes ranged from 5 to 11. Nera Black chicken *TNFRSF1A* gene had the highest values of haplotype diversity and average number of nucleotide difference (0.923 and 3.967 respectively). Frizzle chicken *TNFRSF1A* gene had the lowest values of number of haplotype, haplotype diversity, average number of nucleotide difference, nucleotide diversity, number of polymorphic sites, singleton variable sites and parsimony informative sites (5, 0.733, 1.033, 0.002, 4, 2 and 2 respectively). Haplotype diversity (11), nucleotide diversity (0.009) number of polymorphic sites (21), singleton variable sites (12) and parsimony informative sites (9) were estimated in Naked neck local chickens.

Polymorphism and Genetic Diversity within *zyxin* gene of Four Chicken Genotypes

The results from DnaSP analyses indicated that the selected region (1-1193bp) of the 120 sequences from the four genotypes (Normal feather =38, Naked neck =36, Frizzle feather = 20 and Nera Black =26) had 514 nucleotide sites excluding sites with gaps (678). There were non-variable and variable sites that included 325 singleton variable sites and 104 parsimony informative sites. The haplotype diversity (H_d=0.996) and average number of nucleotide diversity (k=272.5) for all chicken *Zyxin* sequences were higher than the highest values in Frizzle feather local chicken (H_d= 0.857, k=3.143 respectively).

The genetic diversity of the *zyxin* gene in the four chicken genotypes used in this study is presented in Table 2. The number of haplotypes ranged from 5 to 11. The highest haplotype diversity (0.876) and number of haplotype (11) were found in the Normal feather local chicken. The highest average number of nucleotide difference (3.143) and nucleotide diversity (0.00489) were detected in the Frizzle feather local chicken. The Naked neck chicken had the highest values of number of polymorphism sites (15), singleton variable sites and parsimony informative sites (5) while the lowest values of number of polymorphic site (5), singleton variable sites (3), parsimony informative sites (2), nucleotide diversity (0.0016) and average number of nucleotide difference (1.026) were found in the Nera Black. The lowest value of haplotype number (5) was found in the Nera Black and Frizzle feather local chickens while the lowest value of haplotype diversity (0.619) was estimated in the Naked neck local chicken.

Table 1. Genetic diversity of the *TNFRSFA1* gene in the Nigerian Local and Nera Black Chickens

Chicken genotypes	Diversity parameters									
	N	H	H_d	K	π	π_s	π_a	S	SP	PIP
Nera Black	14	9	0.923	3.967	0.008	0.000	0.000	17	10	7
Normal feather	16	6	0.817	2.225	0.005	0.000	0.000	7	2	5
Naked neck	27	11	0.815	3.670	0.009	0.000	0.000	21	12	9
Frizzle feather	16	5	0.733	1.033	0.002	0.000	0.000	4	2	2

N: Number of sequences; H: Number of haplotypes; H_d: haplotype diversity; K: average number of nucleotide differences; π: Nucleotide diversity; π_s: Synonymous nucleotide diversity; π_a: Non-synonymous nucleotide diversity; S: Number of polymorphic sites; SP: Singleton variable sites; PIP: Parsimony informative sites.

Table 2. Genetic diversity of the *zyxin* gene in the Nigerian Local and Nera Black Chickens

Chicken genotypes	Diversity parameters									
	N	H	H_d	K	π	π_s	π_a	S	SP	PIP
Nera Black	13	5	0.628	1.026	0.00160	0.00000	0.000	5	3	2
Normal feather	21	11	0.876	2.300	0.00372	0.00000	0.000	14	10	4
Naked neck	21	7	0.619	2.133	0.00352	0.00000	0.000	15	10	5
Frizzle feather	7	5	0.857	3.143	0.00489	0.00000	0.000	8	4	4

N: Number of sequences; H: Number of haplotypes; H_d: haplotype diversity; K: average number of nucleotide differences; π: Nucleotide diversity; π_s: Synonymous nucleotide diversity; π_a: Non-synonymous nucleotide diversity; S: Number of polymorphic sites; SP: Singleton variable sites; PIP: Parsimony informative sites.

Average Number of Nucleotide Substitutions per site (D_{xy}) of the *TNFRSF1A* Gene

The average number of nucleotide substitutions per site (D_{xy}) of the *TNFRSF1A* gene between the chickens' genotypes is presented in Table 3. The smallest D_{xy} value (0.06542) was estimated between Nera Black and Frizzle feather local chickens while the highest D_{xy} value (0.08133) was found between Naked neck and Normal feather local chickens. Nera Black and Normal feather local chickens; Nera Black and Naked neck chickens had the same value of D_{xy} (0.06729).

Table 3. Average number of nucleotide substitution per site (D_{xy}) between chicken genotypes for *TNFRSF1A*

Genotype	Nera Black	Frizzle feather	Normal feather
Frizzle feather	0.06542		
Normal feather	0.06729	0.07290	
Naked neck	0.06729	0.06716	0.08133

Average Number of Nucleotide Substitutions per site (D_{xy}) of the *Zyxin* Gene

The average number of nucleotide substitutions per site (D_{xy}) of the *Zyxin* gene between chicken genotypes is shown in Table 4. The highest value of D_{xy} (0.65517) was observed between Frizzle feather and Normal feather local chickens while the lowest value of D_{xy} (0.07394) was obtained between Normal feather and Naked neck local chickens.

Table 4. Average number of nucleotide substitution per site (D_{xy}) between chicken genotypes for *zyxin* gene

Genotype	Nera Black	Frizzle feather	Normal feather
Frizzle feather	0.61607		
Normal feather	0.63727	0.65517	
Naked neck	0.59259	0.65010	0.07394

Phylogenetic Relationship between Nigerian Local Chickens and Nera Black Chickens using *TNFRSF1A* Gene Sequences

Phylogenetic relationship of Nigerian local chickens and Nera Black chickens using sequences from *TNFRSF1A* gene is shown in Figure 1. The optimal tree with the sum of branch length = 0.1447 is shown. Nera Black and Frizzle feather lineage are associated with low bootstrap value.

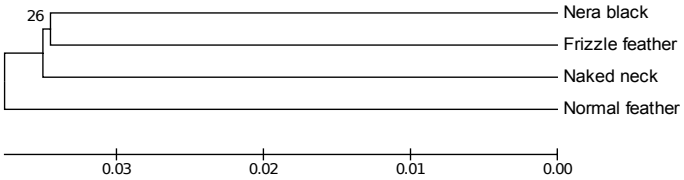

Figure 1. Phylogenetic tree based on consensus sequences of three Nigerian local and Nera

Black chickens *TNFRSF1A* sequences using UPGMA. The tree is drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree which are the number of base substitutions per site.

Phylogenetic Relationship of Nigerian Local and Nera Black Chickens using *zyxin* gene

The evolutionary history was inferred using the UPGMA method. Figure 2 shows a neighbor-joining phylogenetic tree with the bootstrap value for *zyxin* gene sequences representing the major *zyxin* gene sequences lineages from the Nigerian local and Nera black chickens. The optimal tree with the sum of branch length = 2.2571 is shown. Naked neck and Normal feather lineage are associated with high bootstrap value. The phylogenetic analysis did not show support for the basal branching order between the characterised Nera Black, Naked neck and Normal feather lineage.

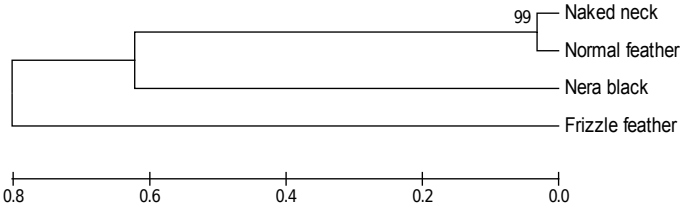

Figure 1. Phylogenetic tree based on consensus sequences of three Nigerian local and Nera

Black chicken *zyxin* sequences using UPGMA. The tree is drawn to scale with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree, which are the number of base substitutions per site.

Nucleotide Synonymous and Non-synonymous Substitutions per site in *TNFSRF1A*

Synonymous and non-synonymous substitutions of *TNFSRF1A* sequences are presented in Table 5. The rate of non-synonymous to synonymous substitutions per site in all genotypes was not significantly different ($p>0.05$). With respect to Naked neck and frizzled feather local chickens, the non-synonymous substitutions per site were greater than the synonymous substitutions per site while the non-synonymous substitutions per site were greater than the synonymous substitutions per site in Nera Black and Normal feather local chickens. The probability of rejecting the null hypothesis of strict-neutrality ($d_N = d_S$) in favour of the alternative hypothesis ($d_N > d_S$) (in the probability column) is shown. Values of p less than 0.05 are considered significant at the 5% level. The test statistic ($d_N - d_S$) is shown in the statistic column. d_S and d_N are the numbers of synonymous and nonsynonymous substitutions per site, respectively. The variance of the difference was computed using the bootstrap method (1000 replicates). Analyses were conducted using the modified Nei-Gojobori.

Nucleotide Synonymous and Non-synonymous Substitutions per site in *zyxin* gene

Nucleotide substitutions per synonymous and non-synonymous sites in *zyxin* gene are presented in Table 6. The rate of non-synonymous to synonymous substitutions per sites in Nera Black and Naked neck local chickens was not significantly different ($p>0.05$) but it was significantly different ($p<0.05$) in the Frizzle feather and Normal feather local chickens. The non-synonymous substitutions per site were approximately equal to synonymous substitutions per site in all chicken genotypes.

Discussion

The results of genetic diversity within each selected genotype for all *TNFRSF1A* sequences were higher than the value in Naked neck chickens. Divergence of the genotypes could be inferred from high genetic diversity of the *TNFRSF1A* gene. The highest haplotype diversity found in Nera Black suggests an abundant genetic diversity at the *TNFRSF1A* locus. This might not be unconnected with the composition of Nera Black being an admixed strain of chicken - Rhode Island Red hybrid breed and higher genetic diversity in Nera Black are most likely useful for its natural selection. The lower haplotype diversity in the Frizzle feather *TNFRSF1A* indicated that Frizzle feather local chicken expansion occurred more recently. The lowest values for haplotype number, nucleotide diversity, average number of polymorphic sites and parsimony informative sites showed that Frizzle feather chickens are less variable at the *TNFRSF1A* locus.

The results of genetic diversity within each selected genotype for all *zyxin* sequences were higher than the values in Naked neck chickens. The highest haplotype number, haplotype diversity, singleton variable sites, number of polymorphic sites and parsimony informative sites showed that *zyxin* gene in Naked neck chickens had the highest genetic diversity within the chickens.

The average number of nucleotide substitutions per sites (D_{xy}) of the *TNFRSF1A* gene between two genotypes is the index of DNA divergence between or among the sequences. The larger the D_{xy} is, the smaller the genetic distance is. The largest D_{xy} (0.081) displayed the earliest differentiation between Normal feather and Naked neck chickens. The lowest D_{xy} (0.065) displayed the most recent differentiation between Frizzle feather and Nera Black.

Table 5. Synonymous and Non-synonymous Substitutions per site of *TNFSRF1A* gene

Genotype	Codons	d_N (+ SE)	d_S (+ SE)	d_N/ d_S	Z-statistic	P value
Nera Black	210	0.0027 + 0.007	0.0042 +0.002	0.643	1.075	0.142
Normal feather	210	0.0017 + 0.001	0.0020 +0.001	0.850	-1.460	1.000
Naked neck	210	0.0036 + 0.007	0.0035 +0.001	1.020	0.423	0.337
Frizzle feather	210	0.0045 + 0.006	0.0040 +0.001	1.125	1.330	0.093

d_N: Non-synonymous substitutions per site; d_S: Synonymous substitutions per site; SE: Standard Error d_N/d_S: Ratio of non-synonymous substitutions per site to synonymous substitutions per site.

Table 6. Nucleotide Substitution per Synonymous and Non-synonymous site in *zyxin* gene

Genotype	Codons	d_N (+ SE)	d_S (+ SE)	d_N/d_S	Z-statistic	P value
Nera Black	210	0.0127 + 0.004	0.0127 +0.003	1.000	1.075	0.412
Normal feather	210	0.0217 + 0.001	0.0219 +0.009	0.990	1.860	0.021
Naked neck	210	0.0610 + 0.015	0.0609 +0.020	1.001	0.223	0.117
Frizzle feather	210	0.0125 + 0.010	0.0125+ 0.008	1.000	1.031	0.035

d_N: Non-synonymous substitutions per site; d_S: Synonymous substitutions per site; SE: Standard Error; d_N/d_S: Ratio of non-synonymous substitutions per site to synonymous substitutions per site.

Based on *zyxin* gene, the largest D_{xy} (0.0655) between Frizzle feather and Normal feather local chickens indicated earliest differentiation compared to other genotypes. This is in contrast to the *TNFRSF1A* gene between Normal feather and Frizzle feather local chickens. The lowest D_{xy} (0.074) displayed the latest differentiation between Normal feather and Naked neck local chickens. *TNFRSF1A* gene might be a better candidate gene for coccidiosis in the crossbred between these two genotypes since higher heterozygosity in their crossbreed will be a good genetic marker for coccidiosis disease tolerance or susceptibility.

Results of phylogenetic analysis of *TNFRSF1A* and *zyxin* genes indicated that the chicken genotypes were clearly separated from one another. In *TNFRSF1A* gene, Normal feather local chickens were the most distant among the chicken populations and this genotype on the evolutionary scale with other genotypes diverged progressively. The second clades consist of Frizzle feather and Nera Black as first sub-cluster while Naked neck local chicken formed the second sub-cluster, respectively. Normal feather local chicken among these chicken populations appeared to be the closest to the origin in evolutionary trend, hence the most remote, as indicated by its distinct and separate cluster. Naked neck local chicken is the most outbred in term of *TNFRSF1A* gene being the farthest from Frizzle feather and Nera Black chickens as a line of descent with all the other three genotypes. The Frizzle feather and Nera black chickens are more closely related evolutionary from the standpoint of the evolutionary tree. This implies that Frizzle feather and Nera Black *TNFRSF1A* gene sequences share a more recent common ancestor than either shared with any of the other genotypes on the phylogenetic tree. In *zyxin* gene sequences, Frizzle feather chickens were the most distant among the chicken populations. The genotypes were divided into two clades with the first clade consisting of only Frizzle feather local chickens as a distinct genotype on the evolutionary scale with other genotypes diverging progressively. The second clade consists of Naked neck and Normal feather chickens as first sub-cluster, respectively. The Frizzle feather local chickens among these chicken populations appeared to be closest to the origin of the evolutionary trend, hence the most remote in terms of *zyxin* gene sequences as indicated by its distinct and separate cluster. Naked neck and Normal feather local chicken populations share a more recent common ancestor than either shares with any of Nera Black or Frizzle feather local chickens on the phylogenetic tree. Phylogenetic analyses of *TNFRSF1A* and *zyxin* gene observed in this study differed from the report of Adeleke (2009), who reported from their preliminary screening of genetic lineage of Nigerian local chickens based on blood protein polymorphisms that Naked neck local chicken population appeared to be closest to the origin in the evolutionary trend and Frizzle feather and Normal feather local chickens shared a more recent common ancestor. The differences observed in these results with Adeleke (2009) might be due to the differences in the genetic markers used in the study. Adeleke (2009) used blood proteins, while in this study only two gene sequences were used.

Generally, synonymous mutations observed in *zyxin* and *TNFRSF1A* are under evolutionary pressure and they may be implicated in coccidiosis disease. Several of the mechanisms by which synonymous mutations alter the structure, function and expression level of proteins have been elucidated. Holla et al. (2009) demonstrated that synonymous polymorphisms can affect messenger RNA splicing, stability and structure as well as protein folding. These changes can have a significant effect on the function of *zyxin* or therapeutic targets and often explain the different responses of individual to a certain medication. Any synonymous base pair changes in *zyxin* or *TNFRSF1A* messenger RNA splicing motif regions can change the splicing patterns of messenger RNA transcripts directly or they can alter the penetrance of concurring mutations elsewhere in the gene. Although Anfinsen's (1973) principle holds that the amino acid sequence of protein alone determines the three-dimensional structure of the protein and mutations which do not alter amino acid residues would not affect the tertiary structure of the protein or its function. Simon et al. (2001) supported Anfinsen's (1973) principle and stated that synonymous mutations were evolutionarily silent because mutations in the genome were selected from the basis of the effect on the fitness of the animal. Computational and experimental studies in recent years suggested that neither Anfinsen's (1973) principle nor Simon et al. (2001) perceptions may be entirely true since non-synonymous and synonymous SNPs within regulatory regions have a greater tendency, relative to their synonymous SNP counterparts, to affect gene behaviour when analysed in isolation.

The ratios of non-synonymous to synonymous substitution (d_N/d_S) of Frizzle feather and Naked neck chicken *TNFRSF1A* gene which were greater than one suggested variation at this gene under positive selection, probably for the recognition of a wide range of infectious agents. Positive selection is a process by which new beneficial genetic variants swing a population. Positive selection in genes can point to the evolutionary basis for difference among breeds within a species (Andreas, 2007). The low ratio of d_N/d_S observed in Nera Black and Normal feather chicken *TNFRSF1A* gene implied balanced selection and the d_N/d_S of *zyxin* gene in all chicken genotypes indicated neutral mutation. This is unique among evolutionary forces in the way that it promotes adaptation. A neutral mutation is a missense mutation that alters the amino acid sequence of the protein but does not change its function. It occurs when one amino acid is replaced by another that is chemically similar or when the affected amino acid has little influence on protein function (Benjamin, 2008) This pattern of evolution (neutral mutation) suggests the action of natural selection on the *zyxin* gene, since neutral polymorphism is not expected to persist very long in a population. Pathogen recognition may provide selection pressure to maintain particular *zyxin* sequences (Minde et al., 2011).

In silico functional analysis using PANTHER revealed relatively low subPSEC and P deleterious values for this mutation, 0 and 0.267 for p.R21K in Naked neck chicken *TNFRSF1A*. These values indicated a likely non-deleterious effect of this missense mutation because the substitution is functionally neutral.

All mutations in *zyxin* protein occurred at positions that do not align to the PANTHER library Hidden Markov Model (HMM), because substitution occurs at positions that do not appear in the multiple sequence alignment of PANTHER library; a subPSEC score was not generated indicating that the mutations observed in *zyxin* protein occurred at positions that were inserted relative to the consensus HMM for the given HMM. These positions were not modelled by the HMMs because they do not appear in most of the related sequences; as a result, substitutions at the inserted positions are not generally likely to be deleterious (Thomas et al., 2006).

Genetic differences exist in *TNFRSF1A* and *zyxin* genes among the four chicken genotypes. Our results support significant diversity at *TNFRSF1A* and *zyxin* loci. This knowledge would be relevant for performing association analysis between *TNFRSF1A* or *zyxin* locus and coccidiosis. Nigerian local chickens, particularly Frizzle feather and Naked neck chickens should be conserved as reservoirs of rare genetic chicken resource and these two genotypes should be encouraged to be integrated into breeding programs.

Conclusion

This study provided the first characterisation of *zyxin* and *TNFRSF1A* genes in Nigerian local chickens and Nera Black chicken. Based on *TNFRSF1A* gene in phylogenetic tree, Nera Black and Frizzle-feathered chickens were generally close to Naked neck chickens but these three genotypes were genetically far from Normal-feathered chickens. With respect to *zyxin* gene in the evolutionary tree, the Naked neck and Normal-feathered local chickens were genetically close to Nera Black, but these three genotypes were genetically far from Frizzle-feathered local chickens. The high haplotype diversity observed in this study will help in designing association study involving samples from genetically distinct chicken population.

Acknowledgments

Our appreciation goes to Federal University of Agriculture, Abeokuta, for funding part of this research under Directorate of Grant Management.

References

Adeleke M.A. (2009). Fertility, Hatchability, Growth performance and blood protein polymorphism of Nigerian indigenous chickens for broiler breed. PhD. University of Agriculture, College of Animal science and Livestock Production, Abeokuta, Nigeria

Adeleke M.A., Peters S.O., Ogunmodede D.T., Oni O.O., Ajayi O.L., Wheto M., Adebambo O.A. (2015). Genotype effect on distribution pattern of maternally derived antibody against Newcastle disease in Nigerian local chickens. Trop Anim Health Prod 47: 391-394

Adenaike A.S., Peters S.O., Adeleke M.A., Fafiolu A.O., Takeet M.I., Ikeobi C.O.N. (2018). Use of discriminant analysis for the evaluation of coccidiosis resistance parameters in chickens raised in hot humid tropical environment. Trop Anim Health Prod 50:1161-1166

Andreas, W. (2007). Rapid detection of positive selection in genes and genomes through variation clusters. Genet 176(4): 2451-2463

Anfinsen C.B. (1973). Principles that govern the folding of protein chains. Science 181: 223-230

Benjamin A.P. (2008). Genetics: A conceptual Approach. W.H. Freeman and Company, New York, USA, pp 500-520

Brunham L.R., Singaraja R.R., Pape T.D., Kejariwal A., Thomas P.D., Hayden M.R. (2005). Accurate prediction of the functional significance of single nucleotide polymorphisms and mutations in the ABCA1 gene. PLoS Genet 1: e83

Fassil B., Adnoy T., Gjoen H.M., Kathle J., Abebe G. (2010). Production performance of dual purpose crosses of two indigenous with two exotic chicken breeds in subtropical environment. Int J Poult Sci 9 (7): 702-710

Groeneveld L.F., Lenstra J.A., Eding H., Toro M.A., Scherf B., Pilling D., Negrini R., Finlay E.K., Jianlin H., Groeneveld E., Weigend S., GLOBALDIV Consortium (2010). Genetic diversity in farm animals—a review. Amin Genet 41(1): 6-31

Hall T.A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Window 95/98/NT. Nucleic Acids Symp Ser 41: 95–98

Holla Ø.L., Nakken S., Mattingsdal M., Ranheim T., Berge K.E., Defesche J.C., Leren T.P. (2009). Effects of intronic mutations in the LDLR gene on pre-mRNA splicing: Comparison of wet-lab and bioinformatics analyses. Mol Genet Metab 96 (4): 245-252

Hong Y.H., Kim E.S., Lillehoj H.S., Lillehoj E.P., Song K.D. (2009). Association of resistance to avian coccidiosis with single nucleotide polymorphisms in the *zyxin* gene. Poult Sci 88 (3): 511-518

Jukes T.H., Cantor C.R. (1969). Evolution of protein molecules. In: Mammalian Protein Metabolism (Munro, H.N. eds), Academic Press, New York, USA, pp. 21–132

Minde D.P., Anvarian Z., Rüdiger S.G.D., Maurice M.M. (2011). Messing up disorder: how do missense mutations in the tumor suppressor protein APC lead to cancer. Mol Cancer 10 (1):101 doi:10.1186/1476-4598-10-101

Moon H.S., Even-Ram S., Kleinman H.K., Cha H.J. (2006). *Zyxin* is upregulated in the nucleus by thymosin β4 in SiHa cells. Exp Cell Res 312:3425-343

Nei, M., Gojobori T. (1986). Simple methods for estimating the numbers of synonymous and non-synonymous nucleotide substitutions. Mol Biol Evol 3 (5): 418–426

Nei M., Kumar S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York USA, pp 16-27

Rezaei, N. (2006). TNF-receptor associated periodic syndrome (TRAPS): An autosomal dominant multisystem disorder. Clin Rheumatol 25:773-777

Schneider S., Roessli D., Excoffier L. (2000). Arlequin (Version. 2.0) A software for population genetic data analysis. University of Geneva, Geneva, Switzerland

Simon A., Dode C., Van der Meer J.W., Drenth, J.P. (2001). Familial periodic Fever and amyloidosis due to a new mutation in the *TNFRSF1A* gene. Am J Med 110: 313–316

Tamura K., Stecher G., Peterson D., Filipski A., Kumar S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis (version 6.0.). Mol Biol and Evol 30: 2725-2729.

Thomas P.D., Campbell M.J., Kejariwal A., Mi H., Karlak B., Daverman R., Diemer K., Muruganujan A., Narechania A. (2003). Panther: a library of protein families and subfamilies indexed by function. Genome Res 13 (9): 2129–2141.

Thomas K.A., Paul D., Guo H., Mi N., Campbell M.J., Muruganujan, A., Lazareva- Ulitsky, B. (2006). Applications for protein sequence function evolution data: RNA/protein expression analysis and coding SNP scoring tools. Nucleic Acids Res 34: 645–650.

Thompson J.D., Higgins D.G., Gibson T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalities and weight matrix choice. Nucleic Acids Res 22: 4673–4680.

Um J.Y., Lee J.H., Joo J.C., Kim K.Y., Lee E.H., Shin T., Hong S.H., Kim H.M. (2005). Association between tumor necrosis factor-alpha gene polymorphism and Sasang constitution in cerebral infarction. Am J China Medicine, 33: 547–557

aCS84_38