

Polymorphism of Ghrelin Genes in three Feather Strains of Nigerian Indigenous Chickens Could Improve the Fitness of Global Chicken

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Abstract

Background: The Nigerian native chicken constitutes the major component of African chicken population. Its ghrelin gene diversity could certainly be associated with traits that can enhance the poultry output necessary for Africa predicted enormous potential for agriculture and support for global food security by 2050. However there is porous information on single nucleotide polymorphism (SNPs) at gene loci of different feather types of chickens.

Methods: 127 blood samples were randomly collected from Nigerian local chicken markets located at 4 zones. Blood sample (0.5 ml) was collected from the wing vein for DNA extraction later. Two pairs of primers (GHRL1 and GHRL2) were used for the study. The SNPs and phylogeny were determined using MEGA software.

Results: The sequence results showed 15 SNPs. The study on nucleotide diversity showed that Nigerian chicken strains varied from 0.4053 in NN to 0.6042 in NF at ghrelin loci. The phylogeny study revealed that the NN was relatively distant from the other two populations, authenticates the normal strain as the wild type chicken and indicates that GHRL 1 gene may have more fitness for tropical climate.

Conclusion: Thus SNPs at ghrelin locus may enhance the fitness traits of global chicken.

Keywords: SNPs; Ghrelin Gene; Growth; Feather; Diversity; Fitness; Global chickens

INTRODUCTION

The Nigerian local chicken has been classified based on major genes of feather distribution comprising naked neck, frizzle feathered and normal feathered (Peters et al. 2008). The Nigerian chicken was reported to be genetically diverse by use of allozymes and microsatellite markers (Adeleke *et al.*, 2011 and Ohwojakpor et al, 2012).

There is probably little doubt that the existence of genetic variation is advantageous to the evolutionary survival of a species. The advantage is impacted is the ability to evolve rapidly and so to meet the needs of the changing environment, both through the course of time and in the colonization of new locality. There are two known factors that bring about continued existence of variation. They include sexual reproduction and outbreeding (Falconer 1970). The three strains of the indigenous chickens is example of an evolutionary measure adopted for survival

of the chicken. The normal, naked neck and frizzled feather are genetic variations observable in the feather gene of the chickens. These must have evolved through time and localization of the wild chicken to meet the changing climate in the tropics.

The significance of genetic variation to nature is further authenticated given the comparatively recent origin of the naturally inbreeding species among the higher plants. This implies that genetic variability is necessary for continued existence of a species over a long period of time.

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This put in another way means that the prevalence of genetic variability among existing species is because those without it have not survived (Falconer 1970). However research has shown that possession of genetic variability does not throw light on the mechanism that maintain it. In a similar way the presence of relatively fewer variations among alleles or species probably reveal the recent origin of such alleles.

Mutation plays a prominent role in restoring genetic variation lost to random drift or selection (Falconer 1970). Therefore the mutation that led to the other chicken feather types (frizzled and naked neck) could probably be restoring genetic variation lost to random drift or selection. The knowledge of the reason for existence of genetic variability is pertinent for the full comprehension of the part mutation played in the course of restoring what is lost to random drift. The existing variation in the feather of chicken could have resulted as a means of protecting chicken from the harsh tropical climate through natural selection. Hence it is implied that what was lost to random drift is restored in the frizzled and naked neck feathered chicken. If selection pressure is zero then the amount of genetic variation would come to equilibrium when its rate of origin was equal to its rate of extinction by random drift. According to the experiment of Clayton and Robertson it would take about 1000 generations for mutation to restore the genetic variation to its original level. This proves that mutation have negligible influence on the response of non-inbred populations to artificial selection (Falconer 1970).

Feather type of chicken is a qualitative or major gene, since the can be studied individually and their values are fairly easily discovered (Khann and Ashok 2000). Two sorts of major genes have been distinguished. Those with more or less severely deleterious effects on fitness and they include nearly all the “mutants” of Mendelian genetics as well as lethal. They are recessive or nearly so in their effects on fitness. The second sort of major genes are those for the antigenic difference. Alleles at these loci are at intermediate frequencies, where they are probably maintained by selection favoring heterozygotes. Their effects on fitness are certainly small enough to be regarded as the “wild type”.

The avian whole genome that was released by Sangar Institute in 2004 has revealed that about 15 million Single nucleotide polymorphisms (SNPs) were found in the avian genome (International Chicken Polymorphism Map Consortium, 2004). Many researchers have demonstrated that SNPs usually occur in non coding region more frequently than in coding region (Barreiro et al., 2008). SNPs in the non-coding region may however, still exert

a significant influence on gene expression or protein turnover rate through their consequences on gene splicing, transcription factor binding and interactions with translational machinery of the cell (Wang et al., 2006). SNPs are good genetic markers (Sherry et al 2001) often occurring where selection is acting and fixating most adaptable genes (Barreiro et al., 2008). SNPs may vary in their adaptation value just as individuals do. Adaptation value of an individual is described as the proportional contribution of offspring to the next generation. Information on SNPs therefore could be significant in breeding probably because it may guarantee genetic gain in the immediate population and the future generations. Such SNPs may be useful in selection indices targeted at creating disease resistant breeds. Animal breeders at the world leading breeding companies have used genomic selection to develop stronger, fitter and healthier birds among elite farm, because genomic selection proved a highly veritable breeding tool for sex – limited traits, enhanced predictive accuracy and increased genetic gain (30-50%) (Aviagen, 2012).

Ghrelin gene was first identified by Kitamura (1998) in the stomach tissue of rat. Chicken ghrelin (cGHLR) gene has been mapped to chromosome 7 (Nei et al., 2004). The chicken ghrelin consists of 5 exon and 4 introns, while the human ghrelin had 4 exons and 3 introns (Nei et al., 2004). The name ghrelin is a Proto-Indo-European language, meaning “grow”. Ghrelin is capable of producing 26 amino acid peptides in chickens and 28 amino acid peptides in mammals. The third amino acid peptide of ghrelin, named serine, is modified with n-octanoyl group. This modification is essential for ghrelin activity. Ghrelin is the first known case of a peptide hormone modified by a fatty acid (Masayasu& Kenji, 2005). Polymorphism of ghrelin gene has been reported among 12 Chinese indigenous chicken breeds and commercial chicken (Li et al., 2006), African (Tswana) chickens (Kgwatalala et al., 2012); and Hubbard flex and Ross 308 exotic chickens (Kaczor et al., 2015). The authors reported varying (18, 23 and 25) number of SNPs sites at ghrelin locus of cDNA (Nei et al. (2004), (Li et al., 2006) and Kgwatalala et al. (2012). Association of ghrelin gene receptor polymorphism and traits were found in chickens by fang et al. (2010). There seems to be paucity of information the on polymorphism of ghreln gene of chicken feather strains of local and exotic chicken and its use in breeding decisions for improvement of the Nigeria chicken. Breeding decision on mutations of ghrelin gene of chicken feather types could help explain the evolutionary role of the SNPs and increase the predictive accuracy and enhance genetic improvement of Nigeria chicken, since ghrelin gene and SNPs have effect on protein turnover, which are expressed as several traits

(growth, reproductive and adaptive value of traits, which are basically proteins.

The objective of this study is to determine the polymorphism of ghrelin gene in three strains of Nigerian chickens and their possible influence on the fitness traits of the global chicken.

MATERIALS AND METHODS

Description of the Breed Studied

A total of 127 Nigerian indigenous chickens (20NN, 30FF and 77NF) were sampled from Nigerian indigenous chicken population. Matured chickens used for the study were collected from the local chicken markets in 4 ecological zones of Nigeria namely, North east (Adamawa), North west (Kebbi), South east (Nsukka) and South west (Abeokuta) The figures below (figure 1a, 1b and 1c) show the three Nigerian chicken strains.

Genomic DNA Samples

Ten divergent individuals in each of 3 indigenous chicken strains, normal feathered (NF), naked neck (NN) and frizzle feathered (FF) were used for SNP scanning of the chickens that have slow growth, low reproduction and favourable meat and egg quality. Natural selection is most prominent in the natural environment, where they are reared. These chickens strains exhibit showed enormous differences in morphological characteristics feather characteristics (distribution and structure) growth rate, body size and productive performance. In the present study 0.5 mls of blood was collected from each individual in EDTA bottles and genomic DNA was extracted from the EDTA-anti coagulated blood.

Primer Design

Two pairs of primers were designed for selective amplification (1000bp) of chicken GHRL gene using reference sequence Genbank Accession No. AY303688. Their nucleotide sequences were as follows;

GHRL1 Forward 5'-CATTTCTAAGCTTTTGCCAGTT -3',

Reverse 5'- CACTGTTATTGTCATCTTCTC-3'.

GHRL2 Forward 5'-ATAAAGTGAATGCAAGAATAGT-3',

Reverse 5'-TGTGTGGTGGGAGTTACTAC-3'.

The annealing temperatures for primer 1 and 2 were 50.25°C and 50.80°C, respectively. The primers were synthesized by Integrated DNA Technologies (USA) and PCR reagents synthesized by Norgen Biotek Corporation (Canada). 10ul of sample was used for PCR reaction.

PCR reaction and Sequencing of PCR product:

The PCR reactions were conducted in a 10ul reaction Volume containing 1ul DNA (template), 0.1ul of each primer,

3ul water and 5ul of 2x master mix (Taq DNA polymerase, dNTPs mixture, magnesium chloride, potassium Chloride and PCR enhancer/ stabilizer) (Norgen Biotek Corporation Canada) on an ABI 2700 (Applied Biosystems, Foster city, A) thermal cycler with the following profile; 94°C initial naturation for 2 minutes, followed by 94°C denaturation for 30 seconds, 40 cycles annealing temperature of 58°C for 30 seconds, 72°C extension for 2 minutes and final extension at 72°C for 5 minutes. The specific PCR products were observed and tested on agarose gel electrophoresis and were used for direct sequencing with ABI 377 by MacroGen Inc (Amsterdam the Netherlands).

DNA Sequencing

30ul of each PCR product was sequenced at MacroGen Inc Amsterdam, The Netherlands, using MacroGen DNA sequence protocol. BLAST analysis was conducted at website <http://www.ncbi.nlm.nih.gov/BLAST/>. At the same time, multiple sequences alignments were performed by the MegAlign program of Lasergene Version 6.0 (DNASTar) software. The SNPs were determined using the sequence alignments program, CLUSTAL W, (<http://www.clustal.org/>), implemented in MEGA software. Phylogeny and sequence similarity (monomorphic and polymorphic sites) of the Nigerian chickens was analyzed with the algorithm (Maximum Likelihood) of MEGA Software.

Statistical Design

Model;

$$Y_{ijk} = \mu + B_i + e_{ijk};$$

Where;

μ : Means;

B_i : Strains effects (NN, NF and FF);

e_{ijk} : individual observations on SNPs, haplotypes and polymorphic site of the indigenous chickens. The data collected were not subjected to Anova, but sequences generated were submitted to MEGA software.

RESULTS

PCR and SNPs at Ghrelin locus of three Nigerian chicken strains

The PCR at GHRL locus of the Nigerian chicken strains are shown in Figures 2 and 3 below. The results of SNPs at ghrelin locus of Nigerian native chickens are showed in table 1 below. This study showed that there were 15 SNPs present at the ghrelin locus chickens of Nigeria. The SNPs were found in the intro, which is the non coding region of the DNA.

Table 1. SNPs at Ghrelin gene locus of three strains of Nigerian native chickens

No	SNP position	Nucleotide	Amino Acid change	Codon	Gene locus	Strains found
1	5	C→T	SER2PHE	None	GHRN 2	NN
2	7	A→G	ASN3ALA	None	GHRN 2	FF
3	12	A→C	ILE4ALA	None	GHRN 2	FF
4	20	T→C	ALA7DEL	None	GHRN 2	NF
5	25	G→A	GLY9ARG	None	GHRN 1	NF
6	31	G→T	ASP9SFP	None	GHRN 1	NF
7	265	A→T	THR89DEL	None	GHRN 2	NF
8	381	G→A	LEU127GLN	None	GHRN 2	NF
9	383	C→T	SER128PHE	None	GHRN 1	NF
10	515	A→T	ASP172PHE	None	GHRN 1	NF
11	892	C→A	LEU298ILE	None	GHRN 2	NF
12	1024	C→T	PRO342SER	None	GHRN 2	NF
13	1036	A→G	ASN346DEL	None	GHRN 2	NF
14	1075	G→C	GLY358ASP	None	GHRN 2	NF
15	1075	G→T	VAL359LEU	None	GHRN 2	NF

Nucleotide and Haplotype diversity

The Nucleotide and Haplotype diversity of three strains (naked neck, normal and frizzle strains) of indigenous chickens of Nigeria are shown in table 2 below. 13 variants were identified at the ghrelin locus of three chicken strains in Nigeria. At the ghrelin locus high nucleotide diversity (0.4053, 0.6042 and 0.5584) and (0.5796, 0.4747 and

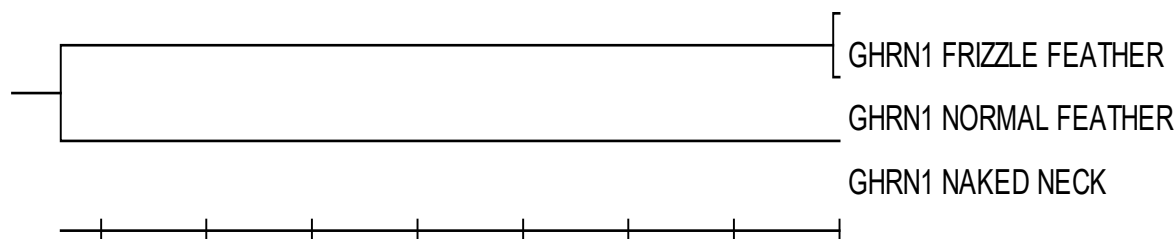
0.4839) were recorded at cGHRL1 and cGHRL2 loci among the three strains of Nigeria indigenous chickens.

However nucleotide diversity of ghrelin gene in three strains of Nigerian chickens ranged from 0.4053 in naked neck to 0.6042 in normal chickens; 0.4747 in normal chickens to 0.5796 in naked neck chickens at cGHRL1 and cGHRL2 loci respectively.

Table 2. GHRL diversity of three strains of Nigerian indigenous chickens

GENES	GHRL 1			GHRL 2		
Genotype	Haplotype diversity	Nucleotide diversity	Sequence conservation	Haplotype diversity	Nucleotide diversity	Sequence conservation
Naked neck	1	0.4053	0.419	1	0.5796	0.246
Normal feather	1	0.6042	0.037	1	0.4747	0.086
Frizzle feather	1	0.5584	0.442	1	0.4839	0.516

Phylogeny

**Figure .** Phylogeny of three strains of Nigerian indigenous chickens

The dendrogram above (Figure 4) revealed that the Normal feathered and the Frizzle feathered populations formed same cluster, indicating a close similarity.

Polymorphic sites of chicken ghrelin in three strains of Nigerian indigenous chickens

Table 3. *Polymorphic sites of chicken ghrelin in three strains of Nigerian indigenous chickens*

Strains	GHRL 1		GHRL 2	
	Polymorphic site	Singleton variable site	Polymorphic site	Singleton variable site
Naked neck	369	369	596	596
Normal feather	537	76	572	215
Frizzle feather	349	349	391	391

Table 3 above presents the polymorphic sites at ghrelin 1 and 2 loci of the three strains of Nigerian native chickens. The polymorphic sites at ghrelin 1 and 2 loci of the three strains of Nigerian native chickens (naked neck, normal feather and frizzle feather) were 369, 537, 349 and 596, 572, 391 respectively. The singleton variable sites ranged from normal feathered to naked neck chickens at GHRL1 and GHRL2 loci respectively.

DISCUSSIONS

The SNPs reported in this study was entirely different from the SNPs reported by Kgwatalala et al. (2012), Li et al. (2006), Nie et al. (2004) and Nie et al. (2005). These 15 SNPs at the ghrelin locus may be novel and might be unique to Nigerian indigenous chickens. Several SNPs found in the study conforms to earlier report that there are about 15 million SNPs in chickens (International Chicken Polymorphism Map Consortium, 2004).

Diversity of nucleotides observed in the current study attest to the fact that indigenous chickens of Nigeria have not been artificially selected in traits of economic importance and hence exhibit greater variability in performance (phenotype) and the underlying genotypes as asserted by Falconer and Mackay (1996).

The results of phylogeny revealed that the Naked neck chicken population was relatively distant from the other two populations supporting earlier observation of Adebambo (2009) and Adeleke et al. (2011), who reported that Nigerian indigenous Normal and Frizzle feathered chicken populations were closely related using microsatellite markers and allozymes. The frizzle gene could have resulted from a mutation at certain some loci in the normal chickens.

The result on polymorphic sites paralleled singleton variable sites in three Nigerian chickens' strain, except in normal feathered chickens indicating that both parameters could measure same effect (polymorphism).

The polymorphic sites ranged from frizzled feathered to normal feather and frizzle feathered to naked neck chickens at GHRL1 and GHRL2 loci respectively.

In conclusion, two primers used in the present study revealed that there was apparent diversity among SNPs of three Nigerian chicken strains. The SNPs markers also effectively detected similarity of sequences between chicken strains. This indicates that they are potential tool for studying genetic relationships. Selection based on SNPs or genomics selection is a veritable tool for genetic progress in poultry, since its results paralleled the results of conventional breeding. The genetic diversity between this subset of SNPs and phylogeny suggest that genomic-based breeding decision or selection index may increase predictive accuracy and improve the egg and meat traits of Nigerian chicken strains. Further, the marked molecular differences between polymorphism at ghrelin gene loci suggests that genetic improvement of the Nigerian chickens warrant separate selection indices. The differentially expressed genetic diversity observed in our study deserves further research.

Wild type of chicken: The result of the amount of polymorphic site recorded at the ghrelin locus of the chickens offers an insight into the older strain of chicken, alternately referred to as the wild strain. Mutation is a mechanism of gene frequency, genotype frequency. it is also one of the possible cause of diversity in polymorphic sites of a gene otherwise called genetic genetic diversity. Recombinant or non-recombinant determine the rate and amount of mutation as well as concerned polymorphic diversity. Time as a factor has significant role to play in the amount of mutant genes. The result implicates the normal strain as the wild type chicken considering the multitude of genetic changes or polymorphism existing at the ghrelin locus of the chickens. The existing polymorphisms at the GHRL loci are indicative of the fact that GHRL1 could have more fitness under the tropical climate. Thus may play significant role in the fitness of the global chicken.

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