ANALYSES OF EXON 10 OF THYROID STIMULATING HORMONE RECEPTOR GENE IN MEAT-TYPE FUNAAB ALPHA CHICKEN GENOTYPES

Oyewoga, A.T, Ozoje, M.O, Durosaro, S.O, Sule, R and Oloko, A.B.
Department of Animal Breeding and Genetics, Department of Animal Nutrition and Department of Animal Physiology. Federal University of Agriculture, P.M.B 2240 Abeokuta, Ogun State. Nigeria.
For Correspondence, Email : oyewogaazeezah@gmail,com, Mobile: 08033934533

ABSTRACT
The thyroid stimulating hormone receptor (*TSHR*) gene is involved in growth regulation, brain development, behaviour and other chemical reactions in the body. This study analysed *TSHR* gene (exon 10) in meat-type FUNAAB Alpha chickens. Blood samples were collected from 32 meat-type FUNAAB Alpha chickens comprising normal feather, naked neck and frizzle feather. Single Nucleotide Polymorphisms (SNPs) in the gene were identified using Codon Code Aligner software (v.10.0.2 DEMO). The selective forces acting on the gene were predicted with MEGA7 and PROSITE software respectively. Two non-synonymous SNPs (614G>A and 720T>G) were identified in normal feather chickens, while one non-synonymous SNP (863G>T) was identified in naked neck chickens. The minor allele frequencies of the SNPs ranged from 0.13 in normal feather to 0.25 in frizzle feather chickens.

Keywords: Polymorphism, Thyroid Stimulating hormones, Naked neck, Frizzle feather and Normal feather.

DESCRIPTION OF PROBLEM

Chicken is a domesticated jungle fowl species, with attributes of wild species such as the Ceylon jungle fowl that are originally. The indigenous poultry chicken represents valuable resources for livestock development especially in developing countries as a result of their extensive genetic diversity which allows for rearing of chicken under varied environmental conditions (1). They are widely distributed in the rural areas of tropical and sub-tropical countries, majority of which are found in the hands of the rural dwellers. Among the major advantages of indigenous chicken are self-reliance, hardiness, adaptation to harsh environments and ability to withstand the minimal management (2). Nigerian indigenous chicken is a dual purpose bird that is raised for meat and egg production in the rural and peri-urban areas of the country (10). Native chicken contributes substantially to the Gross National Product (7). They are kept in small flock and fed on house refuse, homestead pickings, crop residues, herbage etc.

Nigerian indigenous chickens have survived various diseases and various environmental stress (9), which has led to the assumption that indigenous chickens are naturally and generally resistant to diseases (6).

FUNAAB Alpha chicken was developed by improving Nigerian indigenous chickens through crossbreeding and selection (8). Thyroid stimulating hormone is produced and released into the blood stream by the pituitary gland. It controls production of the thyroid hormones, thyroxine and triiodothyronine, by the thyroid gland binding to the receptors located on cells in the thyroid gland. Thyroxine and triiodothyronine are essential to maintaining the body's metabolic rate, heart and digestive functions, muscle control, brain development and maintenance of bones (3). Hence, the aims of the study is to analyse Exon 10 of Thyroid Stimulating Hormone Receptor Gene (TSHR) in FUNAAB Alpha Broiler Chicken,

MATERIALS AND METHODS

Experimental site

The study was carried out at the Poultry Breeding Unit, Department of Animal Breeding and Genetics, Federal University of Agriculture Abeokuta, ($7'10^0$ N and 3^02^0E) situated in Odeda Local Government Area of Ogun State, Nigeria.

The area lies in the South-Western part of Nigeria and has a prevailing tropical climate with a mean annual rainfall of about 1037mm.The vegetation represents an interphase between the tropical rainforest and the derived Savannah (5).

Experimental Animals

A total of 32 blood samples were collected from FUNAAB Alpha broiler chicken at the Poultry Breeding Unit at Federal University of Agriculture, Abeokuta, Nigeria.

Blood sample collection

0.5ml of blood was collected from the brachial vein of the chicken population. The blood was kept in Flinders Technology Associates (FTA) card, labeled appropriately and dried at room temperature before storing in dry paper bags (envelopes).

Genomic DNA Extraction

Genomic DNA was extracted at Biotechnology laboratory of the Department of Animal Breeding and Genetics, Federal University of Agriculture, Abeokuta from 32 FUNAAB Alpha broiler chicken using Zymo research quick-g DNA miniprep kit (catalogue number: D3024) as described: 400µl of genomic lysis buffer containing beta- mercaptoethanol be added to 100µl of whole blood. The mixture was vortexed for 6 seconds and allowed to stand at room temperature for 10 minutes. The mixture was transferred to Zymo-Spin column in a collection tube and centrifuged for one minute at 10,000 xg. The collection tube was discarded with the flow–through and the Zymo-Spin column was transferred to a new collection tube after which 200µl of DNA pre- wash buffer was added to the spin column and centrifuged for one minute at 10,000 xg. Also, 500µl of g-DNA wash buffer was added to the spin column and centrifuged for one minute at 10,000 xg. The spin column was transferred into an Eppendorf tube and 50µl of DNA elution buffer was added to the spin column. The mixture was incubated for 5 minutes at room temperature and centrifuged at 16,000 xg for 30 seconds to elute the DNA. The eluted DNA was stored immediately for further analyses at -4ºC.

RESULTS AND DISCUSSION

Polymorphisms identified in the TSHR (exon 10) gene of FUNAAB Alpha broiler chickens

Three single nucleotide polymorphism in normal feather chickens, 614G>A, 720T>G, 882 T>, were identified in exon 10 of thyroid stimulating hormone receptor gene of FUNAAB Alpha broiler chickens. The two single nucleotide polymorphism (614G>A and 720T>G) were identified in normal feather and amino acid changes to Purine and pyrimidine respectively. Only one single nucleotide polymorphism was identified in naked neck; 863 G>T and pyrimidine was found. Four single nucleotide polymorphism (57 G>A, 73 C>A, 755 G>T and 854 G>T), were identified in frizzle feather and purine, pyrimidine, pyrimidine, and pyrimidine were found respectively.

Exon 10 of TSHR gene in FUNAAB Alpha broiler chickens was found to be highly polymorphic except for a region in normal feather genotype where there is a deletion. In this study both transition and transversion mutations were observed. Both G>A and T>G transition and transversions mutation observed implying that there was no substitution bias in the mutation region. The 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. The minor allele frequencies ranged between 0.13 and 0.25, this measure gives an idea about the variation of genotypes for a given SNP in a given population that is how common that SNP is (4).

The selective force acting on exon 10 of TSHR of FUNAAB Alpha normal feather and naked neck broiler chickens is a Darwinian selection. These individuals then pass the adaptive traits to their offspring and over time these traits become more common in the population, which is a driving force behind evolution in natural selection. This implies that normal feather and naked neck are more common in the population than the frizzle feather. The frizzle feather FUNAAB Alpha broiler chicken is under purifying selection,

which reduces the genetic diversity, both at sites under direct selection and at linked neutral sites. It plays an important role in shaping genomic diversity in natural population. It occurs to preserve biological function and sweeps away deleterious mutation.

Table 1: Polymorphisms identified in the TSHR (exon 10) gene of FUNAAB Alpha broiler chickens

Genotype	*SNP	Minor allele frequency	Reference allele	Type of polymorphism
Normal feather	614G>A	0.13	G	Purine
	720T>G	0.13	T	Pyrimidine
	882T>-.	0.13	T	Indel
Naked neck	863G>T	0.17	G	Pyrimidine
Frizzle feather	57G>A	0.25	G	Purine
	73C>A	0.13	C	Pyrimidine
	755G>T	0.13	G	Pyrimidine
	854G>T	0.13	G	Pyrimidine

*SNPs were named based on the exact position on exon 10

Selective force acting on TSHR (exon 10) gene of FUNAAB Alpha broiler chickens

Selective forces acting on exon 10 of thyroid stimulating hormone receptor gene in FUNAAB Alpha broiler chicken are presented in Table 2. The forces are Darwinian for both normal feather and naked neck while purifying for frizzle feather genotype.

Table 2: Selective force acting on TSHR (exon 10) gene of FUNAAB Alpha broiler chickens

Genotype	dN/Ds	Probability	Type of selection
Normal feather	1.39	0.17	Darwinian
Naked neck	1.01	0.31	Darwinian
Frizzle feather	-0.43	0.67	Purifying

CONCLUSION AND APPLICATION

- Exon10 of TSHR of all the genotypes of FUNAAB Alpha broiler chicken was polymorphic.
- Prediction of selective events revealed that both Darwinian and purifying selective forces were acting on TSHR gene of FUNAAB Alpha broiler chicken.
- Based on the results of this study, frizzle feather chicken can be selected for because it plays an important role in shaping genomic diversity in natural population.

REFERENCES

1. Apuno, A.A., Mbap, S.T. and Ibrahim, T. (2011). Characterization of local chickens (*Gallus gallus domesticus*) in Shelleng and Song local government Areas of Adamawa State, Nigeria. *Agriculture and Biology Journal of North America*. 2 (1): 6-14.
2. Ajayi, F.O. (2010). Nigerian indigenous chickens. A valued genetic resources for meat and egg production. *Asia Journal of Poultry Science*, 4 (4): 164-172.
3. De Groef, B., Geris, K.L., Manzano, J., Bernal, J., Millar, R.P., Abou-Samra, A.B. (2003). Involvement of thyrotropin-releasing hormone receptor, somatostatin receptor

subtype 2 and corticotropin-releasinghormone receptor type 1 in the control of chicken thyrotropin secretion. *Molecular and Cellular Endocrinology*, 203: 33–39. PMID: 12782401

4. Gent, T., Guan, X, and Edward, J.S. (2015). Screening for genes involved in antibody response to sheep red blood cells in the chicken, *Gallus gallus. Poultry science*, 94 10. 3382/ps/pev/224.
5. Goggle Earth, (2018). Map data C2018 Google.
6. Kulube, K. (1990). Small holder rural Poultry production in Zimbabwe. In: *Proceedings of CTA Seminar on Small-holder Rural Poultry Production, Thessaloniki, Greece*, pp 263-269.
7. Momoh, O.M., Ehiobu, N.O. and Nwosu, C.C. (2007). Egg production of Nigeria local chicken ecotypes under improved management. *Proceeding of the 32nd annual conference of Nigerian Society for Animal Production.* 18-21 March. University of Calabar, 278-281.
8. Ogunpaimo, O.J., Wheto, M., Ojoawo, H.T., Adebambo, A.O., Adebambo, O.A. and Durosaro, S.O. (2020). Use of growth models to predict the body weight of FUNAAB Alpha (Fα) broiler, its crossbreds and two other exotic broiler chickens at early stage of growth. *FUDMA Journal of sciences (FJS)*, 4(1), 686-694.
9. Otim, M.O. (2005). Newcastle disease in village poultry: Molecular and Phylogenetic studies of the Virus and Disease Epidemiology. *PhD thesis* Submitted to the Royal Veterinary and Agricultural University (RVAU), Openuhhagen. Denmark, p.140.
10. Sonaiya, E.B. and Olori, V.E. (1990). Family poultry production in south western Nigeria. Proceedings of International workshop held in November 13-16, 1989 at Obafemi Awolowo University, Ile-Ife, Nigeria. Pp 243-247.