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Assessing Non-synonymous Single Nucleotide Polymorphism of IGF-1 Gene Sequence in Three Nigerian Local Chicken Strains

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Local chicken strains – normal feathered, frizzle feathered and naked neck – were used in this study to assess the single nucleotide polymorphism of insulin like growth factor -1 (IGF-1) gene. A total of sixty (60) chickens (twenty (20) from each strain, from which fifteen (15) - five (5) was sampled per strain for blood collection and DNA extraction) were involved in the work. Jena Bioscience Gmbh preparation kit was used in extracting DNA, while the Shine Gene Primers given by:
GTCGGGCTACTTGAGTTACTAC – Forward.
TTGCGCAGGCTCTATCTGCTC - Reverse.
was used to identify genomic DNA for sequencing of the gene (IGF-1). 2% agarose gel was used to assess the DNA purity. Results showed the polymorphism of IGF-1 gene in these strains with the

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neutral (beneficial) variants being more predominant and as such the tendency to influence the expression of more traits. Thus, making IGF-1 a marker of interest in the genomic selection of chicken for development and improvement.

Keywords: Synonymous; gene sequence; single nucleotide polymorphism; IGF-1; local chicken.

1. INTRODUCTION

Insulin – like growth factor (IGF) is similar in structure to insulin. It induces insulin – like metabolic effects in adipose tissues and muscles [1]. Insulin – like growth factor -1 (IGF-1) protein, is a potent mitogen, altered significantly by genotype is essential for stimulating the differentiation of adipocytes, proliferation, differentiation and metabolism of myogenic cell lines in chickens [2].

The polymorphism of the IGF-1 gene promoter region is associated significantly with breast girth [3]. Polymorphism of IGF-I gene is implicated in regulating IGF-I concentration, growth features and many hormones. Whereas its alleles are associated with many yield characteristics such as birth weight, live weight gain, milk yield and fertility [4].

Single nucleotide polymorphisms (SNPs), changes in a DNA sequence occurring as an alteration in the sequence of the genome, involves the substitution of one nucleotide for another and includes about 90% in the differences among individuals as such the best resource in studying population and mapping genomes. They are variations that occur in the genome at single nucleotides level, are present at an appreciable level in any population, can be linked to phenotypes of interest, used to study phylogeny and evolutionary history Lawrie and Massey, [5] and can as well regulate gene expression and or functions [6].

Using SNP markers in genomic selection are of advantage as markers in that they reside in the DNA; directly affecting protein function, for genetic analysis, inherited in a stable form compared to other markers, thus more suitable as selection markers and readily available where needed [7].

IGF-I is a candidate gene for selection programmes that can be done in terms of meat production efficiency [4]. It affects the pattern of muscle and bone mass formation [8] and stimulates growth of the skeletal muscle by increasing protein synthesis rate as such, increasing level of IGF-1 to increase broiler chickens' body weight [1].

It can thus be used as a molecular marker in genomic selection and the development and improvement of chicken strains, as it is ideal to identify, manipulate and cross-breed for improving genetic potential and can enhance the use of molecular markers in livestock species.

This work was therefore carried out to assess the non-synonymous polymorphism of IGF-1 gene.

2. MATERIALS AND METHODS

Sixty local chickens comprising of twenty Frizzle feathered (FR), twenty Naked neck (NN) and twenty Normal feathered (NM) strains sourced from local markets in Uyo, Uruan, Ibesikpo Asutan, Ibiono Ibom, Ikono and Ikot Ekpene Local Government Areas of Akwa Ibom State, Nigeria, were used in this study which was conducted in the poultry unit of the Department of Animal Science, University of Uyo.

Blood samples were collected from fifteen birds – five from each strain and used for molecular analysis.

Jena Bioscience Gmbh preparation kit was used in extracting DNA, while the Shine Gene Primers given by

GTCGGGCTACTTGAGTTACTAC – Forward

TTGCGCAGGCTCTATCTGCTC - Reverse

was used to identify genomic DNA for sequencing of the gene (IGF-1). 2% agarose gel was used to assess the DNA purity.

Bioinformatics analysis was carried out on the gene sequence using the Protein Variant Effect Analyzer (PROVEAN) for computational functional analysis and non-synonymous mutation. Choi et al., [9] and the Tajima test where the P- distance method is utilized in the computation of evolutionary distances according to Nei and Kumar [10], while the evolutionary analysis was performed with MEGA7 [11].

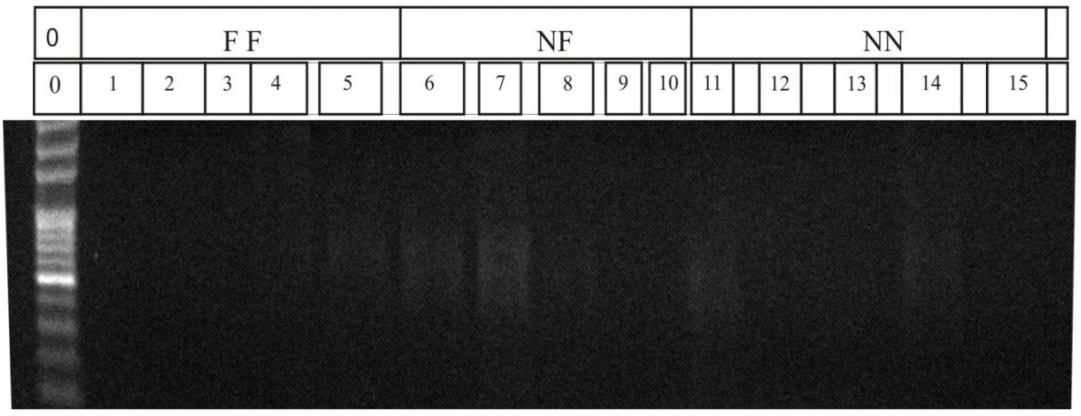
3. RESULTS AND DISCUSSION

The DNA extraction process using 1% agarose gel electrophoresis was successful as a crucial and essential step in isolating the IGF_1 gene. The results, obtained through PCR technology (Fig. 1), demonstrated the successful amplification of the targeted fragment of the IGF_1 gene by electrophoresis on 1.5% agarose gel, resulting in the desired fragment of the IGF_1 gene.

The results for the non-synonymous Single Nucleotide Polymorphism (nsSNP) of Insulin growth Factor 1 (IGF-1) gene for the frizzle feathered, normal feathered and naked neck strains are presented in Table 1. It has a total of twenty (20) variants with four (4 – 20%) of the variants - F364Q, L412V, Q711L and V932Q - being deleterious, while the other sixteen (16 - 80%) variants are beneficial for the frizzle feathered strain, three (3 – 15%) variants - A74K, C17R and D511 - were shown to be deleterious and the remaining seventeen (17 – 85%) beneficial for the normal feathered strain and two (2 – 10%) variants – Y163K and T195G – being deleterious (harmful), while eighteen (18 – 90%) were beneficial for the naked neck strain. These variant forms of the gene, known as short nucleotide polymorphism, according to Koopae and Koshkoiyeh, [7] is as a result of variations in the DNA that occurs as a result of nucleotide base being changed within a sequence in the gene's protein coding region. This results in the substitution of amino acid in the protein which results in neutral/ beneficial or deleterious/ harmful nature of the gene and thus will affect the function of the particular variant of the gene. This is in agreement with Yang et al. [12] and Wang et al. [13].

The variants that resulted from the substitution of amino acid were both deleterious (harmful) and neutral (beneficial) with respect to the three local strains of chicken. The neutral or beneficial amino acid substitution helps keep the stability of cells and tissues structurally. It affects positively the functions proteins play in hormonal and other stimulants functions as well as signal transduction of visual. The harmful cause change which alters the role of protein thus leading to disease susceptibility as metabolic cycles is disrupted [14]. This could result in enzyme activity being modified, structures of protein destabilized or interactions of protein being disrupted [15]. Both scenarios equally interfere with (cause changes in) protein functions [16], depending on the model with which such changes are assessed (Dang et al.,2022), thus altering the outcome of metabolical processes.

The beneficial variants mean protein function was not impaired. The function of SNP is significant in the sense that synonymous mutations especially in regions for coding can function either on its own or in conjunction with various mutations to influence stability and translation of mRNA within the same transcript, leading to functional effects [6]. The analysis of the gene sequence shows that IGF-1 gene is polymorphic with naked neck strain having the lowest number of deleterious but more neutral/beneficial variants followed by the normal feathered strain and the frizzle feathered strain in that order an indication of the fact that protein function will not be impaired. This is in agreement with Wheto et al., [17] who reported polymorphism in frizzle feathered chicken in their work.



F F= FRIZZLE FEATHERED, NF=NORMAL FEATHERED, NN= NAKED NECK, 0 = CONTROL, 123.....15= LADDERS

Fig. 1. Gel electrophoresis of IGF -1 gene

Table 1. Analysis of non – synonymous Short Nucleotide Polymorphism of Insulin Growth Factor-1

Frizzled Feathered			Normal Feathered			Naked Neck		
Variants	**PROVEAN Score**	**Prediction**	**Variants**	**PROVEAN Score**	**Prediction**	**Variants**	**PROVEAN Score**	**Prediction**
K2D	-0.074	Neutral	A10P	-0.010	Neutral	R2V	-0.022	Neutral
K3L	-0.821	Neutral	V31L	-0.210	Neutral	R3S	-0.912	Neutral
G11F	-0.101	Neutral	A71L	-1.101	Neutral	L22V	-0.112	Neutral
Q31L	-1.101	Neutral	G12A	-0.534	Neutral	L22E	-0.178	Neutral
Q31E	-2.01	Neutral	A25L	-0.378	Neutral	D30S	-0.652	Neutral
A42G	-0.289	Neutral	G82L	-0.445	Neutral	V52K	-0.233	Neutral
A42T	-0.031	Neutral	G36P	-0.310	Neutral	E62N	-1.100	Neutral
T82N	-0.542	Neutral	C40R	-0.065	Neutral	E62R	-0.230	Neutral
N112L	-0.078	Neutral	D44L	-0.020	Neutral	E81K	-0.001	Neutral
K203T	-0.971	Neutral	D44Q	-1.230	Neutral	V113S	-0.023	Neutral
K239V	-1.110	Neutral	A74K	-4.450	Deleterious	K139A	-0.745	Neutral
K231Q	-0.091	Neutral	P35T	-2.113	Neutral	Y163K	-2.573	Deleterious
N319P	-0.456	Neutral	P95L	-2.000	Neutral	T195G	-4.902	Deleterious
F364G	-0.021	Neutral	R56S	-0.058	Neutral	D242T	-0.216	Neutral
F364Q	-3.672	Deleterious	C17R	-6.591	Deleterious	V267E	-0.020	Neutral
L412V	-4.449	Deleterious	C57F	-1.043	Neutral	F308L	-0.322	Neutral
Q711L	-5.571	Deleterious	E97T	-1.758	Neutral	S345F	-0.311	Neutral
Q723V	-1.578	Neutral	E88F	-0.377	Neutral	M431V	-1.100	Neutral
H823L	-1.350	Neutral	L11H	-1.059	Neutral	V601L	-0.903	Neutral
V932Q	-4.053	Deleterious	D51I	-3.134	Deleterious	K515R	-2.411	Neutral

Default threshold = -2.5, PROVEAN Score ≤ -2.5 = Deleterious, ≥ -2.5 = Neutral. G=Glycine, A=Alanine= Leucine, M=Methionine, F= Phenylalanine, W= Tryptophan, Q= Glutamine, E= Glutamic Acid, S=Serine, P=Proline, V= Valine, Y= Tyrosine, R= Arginine, N= Asparagine, T= Threonine, C= Cysteine, D= Aspartic Acid, H=Histidine, I= Isoleucine, K=Lysine

Table 2. Results of Tajima's selection test

Strains	M	S	P_S	Θ	Π	D
Frizzled	3	193	0.9896	0.546	0.874	-5.4767
Normal	3	196	1.0000	0.535	0.747	7.4511
Naked	3	196	1.0000	0.523	0.832	6.7444

M - Number of Sites, Ps - S/m, D - Tajima test for selection, π - nucleotide diversity, S - Number of segregating sites, Θ - Ps/ a1

The more beneficial variants leave room for the prevalence of many forms of the gene and as such more functional sites which allow for more expression of traits influenced by the gene in its various forms. The naked strain, which had more beneficial variants, therefore showed the tendency to influence beneficial (positive) mutations will lead to functional effects as the stability translation of mRNA is influenced. This is in agreement with Berg et al, [18].

These variations leave room for the gene, in its numerous variant forms especially the beneficial ones – 16 for frizzle feathered, 17 for normal feathered and 18 for naked neck, to affect production performance of the birds by being associated with the expression of many traits. This is in line with the work of Fouda et al, [19].

The polymorphism of IGF-1 has earlier been reported to be linked with the expression of some performance characters of numerous animals by many authors – growth traits [2,19-23] all in chicken, carcass component Karabag et al., [24] in quail as well as growth trait parameters in sheep and goats [25,26,4].

The result in this study tends to point to the fact that polymorphism of the IGF -1 gene will enhance performance with the presence of more beneficial variants and as such studying the polymorphism (nsSNP) enhances an understanding of metabolic pathways especially as it relates to growth and production performance of animals.

Tajima's selection test results are shown in Table 2. Frizzle feathered strain had the lowest number of segregating sites (193) but with the highest nucleotide diversity (0.874), while the naked neck and normal feathered both had a higher number of segregating sites (196) but with the naked neck having a lower nucleotide value (0.832) and the normal feathered having the lowest nucleotide value (0.747). The normal feathered strain had the highest D value (7.4511) while the naked neck had a D value of 6.7444 and the frizzle feathered the lowest D value (-5.4767). The frizzle strain had a negative D value whereas the normal and naked neck strains had positive values. Tajima's (D) test for selection of the three strains of local chicken, showed a positive D - value for the normal feathered and naked neck strains which showed that levels of polymorphisms frequencies are low showing decreased population or a selection that is balanced [27,28]. However, the frizzle feathered strain had a negative D - value which is an indication of low frequency polymorphism being in excess signifying the expansion of, as well as, purifying selection. A D - value > 0 implies balancing selection with an attendant sudden population contraction while a D – value < 0 implies a recent selective sweep with an attendant population expansion. A selection that is associated with the substitution of amino acid that is non-deleterious is positive and that it cannot be overcome by purifying selection. This implies that selection against non-synonymous polymorphism changes the DNA because of their harmful effect.

The positive D-value for the normal feathered and naked neck strains respectively implied a balanced or sudden population contraction among the strains over time. Whereas the negative D-value for the frizzle feathered strain implied a bottle neck (sweeping) selection with a resultant expansion in population among the strain over time and is in accordance with the works of Nielsen et al.,[27]; Yeh and Contreras,[28].

Nucleotide diversity, which measures genetic diversity, is high for the frizzle feathered and naked neck strains [29,30]. It shows the presence of a wide variety of different traits among the strains. It is important because it gives strains a better chance of survival and if lost (as in when populations get smaller and isolated) reduces the ability of the strains to adapt and survive.

4. CONCLUSION

In conclusion, the polymorphism of the IGF -1 gene resulting in its variant forms makes it a marker of interest in molecular and genomic selection of chicken and other animals for growth traits.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author(s) do hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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