MedCrave
Step into the World of Research
International Journal of Molecular Biology: Open Access

Research Article

Open Access
CrossMark

Analysis of insulin growth factor (IGF-1) gene in some selected monogastrics: An Insilico approach

Abstract

An insilico analysis of insulin growth factor (IGF-1) gene in some selectected monogastrics was analyzed using fifteen amino acid sequences retrieved from the National Center for Biotechnology and Information genebank. The sequence accession numbers are NP_990363, XP_015132626, XP_015132625, AAQ77244 and AGG38009 (chicken) NP_999337, AEV40680, XP_005654025, AAD00174 and XP_005670114 (pig) XP_010722867, XP_010711104, XP_010719115, XP_010720267 and XP_010711104 (turkey). The results of functional analysis of non-synonymous single nucleotide polymorphism (nsSNP) revealed that the amino acid substitution variants for the chicken showed the variants (K47V, N51D, V55A, D69H, F73L, L78S, T82K and L85S) appeared as deleterious while the remaining variants appeared neutral. The nsSNP of pig variants (P35G, R40C, L59P, T53P, R71C, F75C T79E and F88E) appeared deleterious while the remaining variants returned neutral. The nsSNP of turkey variants (H11L, I15D, S19G, L29N, Q33H, K43L and V62A) returned neutral while the rest of the variants returned deleterious. The result of Estimates of Evolutionary Divergence between Sequences of the species (chicken, pig and turkey) revealed that the average nucleotide substitutions per site (Dxy) value recorded for chicken and pig (0.948), chicken and turkey (0.943) and pig and turkey (0.947). This implies that the three species are genetically distant. The evolutionary relationship shown that the species intermingle, which is an evidence of the long-term evolutionary persistence of the locus while the close similarity of a gene among the species may be ascribed to recent separation in evolutionary process and/or similar selection pressure which the ruminants have suffered during evolution. The study concluded that information emanating may be relevant in developing a molecular maker for selection in chicken pig and turkey and also as guide for subsequence dry and wet laboratory experiment.

Keywords: factor, gene, growth, insulin

Volume 3 Issue 2 - 2018

Dauda A,[1] Saul S,[2] Yaska JA,[3] Malgwi IH[4]

[1]Department of Animal Science, University of Calabar, Nigeria
[2]Department of Animal Science, University of Maiduguri, Nigeria
[3]Department of Animal Science, University of Agriculture, Nigeria
[4]Faculty of Agricultural and Environmental Sciences, Kaposvar University, Hungary

Correspondence: Dauda A, Department of Animal Science, University of Calabar, Nigeria, Email ayubadauda87@gmail.com

Received: February 06, 2018 | **Published:** April 23, 2018

Introduction

The use of molecular marker-assisted selection has proven to be efficient and lead to the improvement in production performance in animals.[1] The ability of meat production is closely associated with muscle growth. Recent researches on polypeptides growth factors have identified several growth factors such as insulin growth factors (IGFs), epidermal growth factor, transforming growth factor and platelet-derived growth factor as modulators of muscle.[2,3] Insulin-like Growth Factor 1 (IGF-1) gene has been described in several researches as a candidate gene for growth.[4] It regulates differentiation including the maintenance of differentiated function in numerous tissues and in specific cell types.[5] IGF-1 also stimulates the anabolic and mitogenic activity of growth hormone in various tissues.[6] The primary source of circulatory IGF-1 is the liver however; it is also produced locally in a tissue specific manner.[7] Recent advances in high-throughput technologies have generated massive amounts of genome sequence and genotype data for a number of species. The method to identify functional synonymous polymorphism sequence (SNPs) from a pool, containing both functional and neutral SNPs is challenging by experimental protocols.[8] Therefore, computational predictions have become indispensable for evaluating the disease-related impact of non-synonymous single-nucleotide variants discovered in exome sequencing.[9] A number of computational methods have been developed to predict the functional effect of a non-synonymous single-nucleotide polymorphism (nsSNP) and a single-nucleotide change in a protein-coding region of a gene that causes an amino acid substitution (AAS) in the resulting protein.[10] Many such methods have their roots in molecular evolution, as they use information derived from multiple sequence alignments. Most computational prediction tools for amino acid variants rely on the assumption that protein sequences observed among living organisms have survived natural selection. Therefore, evolutionarily conserved amino acid positions across multiple species are likely to be functionally important, and amino acid substitutions observed at conserved positions will potentially lead to deleterious effects on gene functions.[11] Therefore, this study was design to look at non-synonymous single nucleotide polymorphism, species diversity and evolutionary relationship of igf-1 gene in some selected monogastrics animals.[12]

Materials and Methods

A total of fifteen (15) insulin growth factor-1 nucleotide sequences comprising chicken (5), pig (5) and turkey (5) were retrieved from the Gen Bank (NCBI) (www.ncbi.nlm.nih.gov). The Gen bank accession numbers of the sequences were NP_990363, XP_015132626, XP_015132625, AAQ77244 and AGG38009 (chicken) NP_999337, AEV40680, XP_005654025, AAD00174 and XP_005670114 (pig) XP_010722867, XP_010711104, XP_010719115, XP_010720267 and XP_010711104 (turkey). Sequences alignment, translation and comparison of the) insulin growth factor-1 gene of the various species

was done with Clustal W as described by using IUB substitution matrix, gap open penalty of 15 and gap extension penalty of 6.66**.** In silico functional analysis of insulin growth factor-1 gene missense mutations was obtained using PROVEAN (Protein Variant Effect Analyzer) with threshold value of -2.5. PROVEAN collects a set of homologous and distantly related sequences from the NCBI NR protein database using BLASTP (ver.2.2.25) with an E-value threshold of 0.1. The sequences were clustered based on a sequence identity of 80% to remove redundancy using the CD-HIT program (ver.4.5.5).[13] If the PROVEAN score is smaller than or equal to a given threshold, the variation is predicted as deleterious.

The numbers of amino acid differences per site from between sequences are shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (1000 replicates). The analysis involved 15 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 293 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.[9] The evolutionary history was inferred using the Neighbor-Joining method.[14] The optimal tree with the sum of branch length=14.71812433 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.[15] 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. The evolutionary distances were computed using the Poisson correction method[16] and are in the units of the number of amino acid substitutions per site. The analysis involved 15 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 293 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.[9]

Results

The results of Functional analysis of coding nsSNP of the insulin growth factor-1 gene of chicken, pig and turkey are presented in Table 1,2 and 3 respectively. The result of nsSNP of chicken showed the variants (K47V, N51D, V55A, D69H, F73L, L78S, T82K and L85S) returned as deleterious while the remaining variants returned neutral. The nsSNP of pig variants (P35G, R40C, L59P, T53P, R71C, F75C T79E and F88E) returned deleterious while the remaining variants returned neutral. The nsSNP of turkey variants (H11L, I15D, S19G, L29N, Q33H, K43L and V62A) returned neutral while the rest of the variants returned deleterious. The result of Estimates of Evolutionary Divergence between Sequences of the species (chicken, pig and turkey) is presented in Table 4. The upper diagonal represents standard error estimate(s) while the lower diagonal is the average genetic distances between species which is also known as The average nucleotide substitutions per site (Dxy). The Dxy value recorded for chicken and pig (0.948), chicken and turkey (0.943) and pig and turkey (0.947). Figure 1: showed Evolutionary relationships of Insulin growth factor gene of chicken, pig and turkey. The figure showed some intermingling between species of chicken and pig and then pig and turkey.

Table 1 Functional analysis of coding nsSNP of the insulin growth factors-1 gene of chicken using PROVEAN

Variants	PROVEAN Score	Prediction
A1 1I	-0.234	Neutral
G15S	-0.744	Neutral
L18A	-1.102	Neutral
A22D	-1.097	Neutral
L25Q	-1.793	Neutral
T28G	-0.634	Neutral
V37Q	-2.403	Neutral
H44Y	-0.904	Neutral
K47V	-2.572	Deleterious
N51D	-3.094	Deleterious
V55A	-3.314	Deleterious
S65K	-1.34	Neutral
D69H	-3.7	Deleterious
F73L	-2.515	Deleterious
L78S	-4.972	Deleterious
T82K	-4.972	Deleterious
L85S	-4.497	Deleterious
L93I	-0.1657	Neutral

Default threshold is −2.5, that is; Variants with a PROVEAN score equal to or below −2.5 are considered "deleterious" while Variants with PROVEAN score above −2.5 are considered "neutral". G=glycine, A=Alanine, L=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.

Citation: Dauda A, Saul S, Yaska JA, et al. Analysis of insulin growth factor (IGF-1) gene in some selected monogastrics: An Insilico approach. *Int J Mol Biol Open Access*.2018;3(2):68–71. DOI: 10.15406/ijmboa.2018.03.00053

Table 2 Functional analysis of coding nsSNP of the insulin growth factors-1 gene of pig using PROVEAN

Variants	PROVEAN Score	Prediction
P10A	**-0.096**	Neutral
L13V	-0.543	Neutral
L17A	-1.568	Neutral
A21G	-1.049	Neutral
L25A	-1.311	Neutral
T28G	-0.388	Neutral
P35G	-4.021	Deleterious
R40C	-6.175	Deleterious
Q44D	0.068	Neutral
K47A	-1.35	Neutral
T53P	-3.672	Deleterious
L59P	-5.471	Deleterious
S65R	-1.598	Neutral
R71C	-4.449	Deleterious
F75C	-6.495	Deleterious
T79E	-3.034	Deleterious
F88E	-6.789	Deleterious

Default threshold is −2.5, that is; Variants with a PROVEAN score equal to or below −2.5 are considered "deleterious" while Variants with PROVEAN score above −2.5 are considered "neutral". G = glycine, A = Alanine, L = 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.

Table 3 Functional analysis of coding nsSNP of the insulin growth factors-1 gene of turkey using PROVEAN

Variant	PROVEAN Score	Prediction
H11L	0.062	Neutral
I15D	1.029	Neutral
S19G	0.66	Neutral
G25L	-3.933	Deleterious
L29N	-2.36	Neutral
Q33H	-0.656	Neutral
H38V	-3.411	Deleterious
K43L	-1.75	Neutral
R47G	-2.858	Deleterious
R52C	-4.478	Deleterious
L58D	-4.834	Deleterious
V62A	-1.824	Neutral
A68Y	-3.475	Deleterious
T72D	-2.936	Deleterious
C76Q	-5.955	Deleterious
D82N	-3.248	Deleterious
A86L	-3.063	Deleterious
V90S	-4.536	Deleterious

Default threshold is −2.5, that is; Variants with a PROVEAN score equal to or below −2.5 are considered "deleterious" while Variants with PROVEAN score above −2.5 are considered "neutral". G=glycine, A=Alanine, L=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.

Table 4 Estimates of evolutionary divergence between sequences of the species

	Chicken	Pig	Turkey
Chicken		0.009	0.009
Pig	0.948		0.010
Turkey	0.943	0.947	

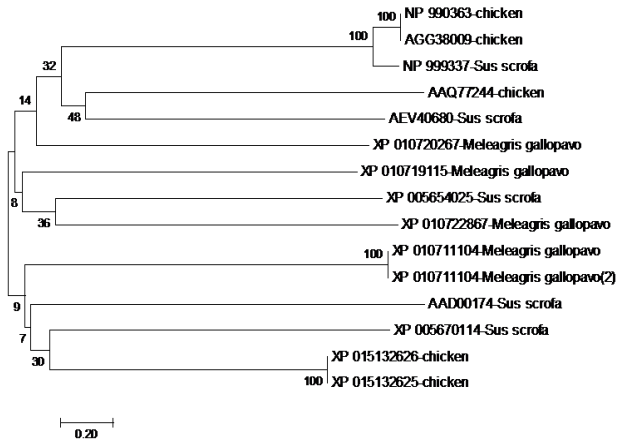

Figure 1 Evolutionary relationships of Insulin growth factor gene of chicken, pig and turkey.

Discussion

Insulin-like Growth Factor-1 (IGF-1) gene has been described in several researches as a candidate gene for growth.[3] In this study the neutral/beneficial amino acid substitution of chicken, pig and turkey are those substitution that do not impaired the amino acid and biological process in the cells while those that appeared deleterious impaired with the protein-protein interaction, protein folding, active site, protein solubility or stability which may lead to disease susceptibility. The neutral/beneficial nsSNP substitution may give hope for future genetic improvement for chicken, pig and turkey at IGF-1 gene locus.[17] This is due to the fact that nsSNPs have been reported to be linked to economically important traits and disease development. [18] Amills et al.[19] and Zhou et al.[20] noted positive association between IGF-1, SNP and average daily weight in poultry. While Fang et al.[1] noted a significant correlation between IGF-1 polymorphism and egg production in poultry. The information from amino acid substitution of IGF-1 gene might be relevant in increase the number of beneficial allele and to give caution of disease allele. Noted the prediction of SNPs status is promising in modern genetics analysis and breeding programmes as they have been used to identify those animals with higher breeding value. [21] Since the aim of animal breeding is to select individuals that have high breeding values for traits of interest as parents to produce the next generation and to do so as quickly as possible.[22] The average genetic distance Dxy is an index of divergence between and among species, where Dxy=distance between sequence x and sequence y. The higher the value of Dxy the far apart the species are, by implication, higher values have lesser ortholog and more paralog and vice versa.[18] The larger the Dxy value, the greater the genetic distance while the smaller the Dxy value the closer the genetic distance between the species.[20] This is an indication that chicken, turkey and pig are genetically distant. The evolutionary relationship of nucleotide sequences of chicken, pig and turkey

Citation: Dauda A, Saul S, Yaska JA, et al. Analysis of insulin growth factor (IGF-1) gene in some selected monogastrics: An Insilico approach. *Int J Mol Biol Open Access.* 2018;3(2):68–71. DOI: 10.15406/ijmboa.2018.03.00053

revealed some presence of many alleles at a particular IGF-1 locus which is an evidence of the long-term evolutionary persistence at the locus. This is suggested by the frequency with which alleles in one species are more closely related to the alleles in a closely related species than to the other alleles in the same species.[23] The information emanating from this study could be exploited in improving the native chicken, pig and turkey. The close similarity of a gene among the species may be ascribed to recent separation in evolutionary process and/or similar selection pressure which the species have suffered during evolution.[24]

Conclusion

The study concluded that IGF-1 gene is polymorphic gene in chicken, turkey and pig that has many mutations which revealed non-synonymous single nucleotide polymorphism of both neutral and deleterious amino acid substitution variants. The genetic divergence revealed that the three species are distantly related genetically. Although the evidence from evolutionary relationship that showed some level of similarities might be from long term evolutionary history and selection pressure which the species might have undergo. This new typing tool may bring insight into developing maker for IGF-1 gene.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

References

1. Fang LH, Zhu W, Chen K, et al. Associations between GHR and IGF-1 gene polymorphisms and reproductive traits in wenchang chickens. *Turk J Vet Anim Sci.* 2008;32:281–285.
2. Florini JR, Ewton DZ, Coolican SA. Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr Rev*. 1996; 17:481–517.
3. Duclos MJ. Regulation of chicken muscle growth by insulin-like growth Factorsa. *Ann N Y Acad Sci*. 1998;839:166–171.
4. Al Hassani AS, Al Hassani DH, Abdul Hassan IA. Association of insulin-like growth factor-1 gene polymorphism at 279 position of the 5'UTR region with body Weight traits in broiler chicken. *Asian Journal of Poultry Science.* 2015;9:213–222.
5. Werner H, Adamo M, Roberts CT, et al. Molecular and cellular aspects of insulin-like growth factor action. In: Litwack G, editor. *Vitamins and Hormones*. Oxford; 1994. p.1–58.
6. Laron Z. Insulin-like growth factor 1 (IGF-1): a growth hormone. *Mol Pathol.* 2001;54(5):311–316.
7. Etherton TD. Somatotropic function: the somatomedin hypothesis revisited. *J Anim Sci*. 2004;82:239–244.
8. George PDC, Rajasekaran R, Sudandiradoss C, et al. A novel computational and structural analysis of nsSNPs in CFTR gene. *Genomic Med*. 2008;2(2):23–32.
9. Liu L, Kumar S. Evolutionary balancing is critical for correctly forecasting disease associated amino acid variants. *Mol Biol Evol*. 2013;30(6):1252–1257.
10. Zemla D, Kostova T, Gorchakov R, et al. GeneSV- an approach to help characterize possible variations in genomic and protein sequences. *Bioinform Biol Insights*. 2014;8:1–16.
11. Choi Y, Sims GE, Murphy S, et al. Predicting the functional effect of amino acid substitutions and indels. *PLoS One*. 2012;**7**(10):e46688.
12. Zhang C, Zhang W, Luo H, et al. A new single nucleotide polymorphism in the IGF-I gene and its association with growth traits in the Nanjiang Huang goat. *Asian-Aust J Anim Sci*. 2008;21(8):1073–1079.
13. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol.* 2016;33(7):1870–1874.
14. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol Biol Evol*. 1987;4(4):406–425.
15. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*. 1985;39(4):783–791.
16. Zuckerkandl E, Pauling L. Evolutionary divergence and convergence in proteins. In: Bryson V, Vogel HJ, editors. *Evolving Genes and Proteins*. New York: Academic Press; 1965. p. 97–166.
17. Bibinu BS, Yakubu A, Ugbo SB, et al. Computational molecular analysis of the sequences of bmp15 gene of ruminants and non-ruminants. *Open Journal of Genetics*. 2016;6(2):39–50.
18. Vincent ST, Momoh OM, Yakubu A. Bioinformatics Analysis of Beta Casein Gene in Some Selected Mammalian Species. *Research Opinions in Animal and Veterinary Sciences*. 2014;4(10):564–570.
19. Amills MN, Jimenez D, Villalba MT, et al. Identification of three single nucleotide polymorphisms in the chicken insulin-like growth factor 1 and 2 genes and their associations with growth and feeding traits. *Poult Sci*. 2003;82(10):1485–1493.
20. Kang JF, Li XL, Zhou RY, et al. Bioinformatics analysis of lactoferrin gene for several species. *Biochem Genet*. 2008;46(6):312–322.
21. Tariq AM, Al Shammari AS, Al Muammar MN, et al. Evaluation and identification of damaged snps in col1a1 gene involved in osteoporosis. *Arch Med Sci*. 2013;9(5):899–905.
22. Dekkers JCM. Application of genomics tools to animal breeding. *Curr Genomics*. 2012;13(3):207–212.
23. Takeshima S, Chen S, Miki M, et al. Distribution and origin of bovine major histocompatibility complex class II DQA1 genes in Japan. *Tissue Antigens*. 2008;72(3):195–205.
24. Sun Y, Zhang X, Xi D, et al. Isolation and cDNA characteristics of MHC-DRA genes from Gayal (*Bos frontalis*) and gaytle (*Bos frontalis*×*Bostaurus*). *Biotechnol Biotechnol Equip.* 2015;29(1):33–39.