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 selected 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_010711004, 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, P71C, F73C, 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 marker for selection in chicken pig and turkey and also as guide for subsequent dry and wet laboratory experiment.

Keywords: factor, gene, growth, insulin

Introduction
The use of molecular marker-assisted selection has proven to be efficient and lead to the improvement in production performance in animals. 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. Insulin-like Growth Factor 1 (IGF-1) gene has been described in several researches as a candidate gene for growth. It regulates differentiation including the maintenance of differentiated function in numerous tissues and in specific cell types. IGF-1 also stimulates the anabolic and mitogenic activity of growth hormone in various tissues. The primary source of circulatory IGF-1 is the liver however; it is also produced locally in a tissue specific manner. 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. Therefore, computational predictions have become indispensable for evaluating the disease-related impact of non-synonymous single-nucleotide variants discovered in exome sequencing. 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. 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. 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.

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_010711004, 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). 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. The evolutionary history was inferred using the Neighbor-Joining method. 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. 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 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.

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 |
|----------|---------------|------------|
| A11I     | -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     | -1.43        | 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.

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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    |
| T33P     | -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”.

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

| Variants | PROVEAN Score | Prediction |
|----------|---------------|------------|
| F88E     | -5.471        | Deleterious|
| T79E     | -3.034        | Deleterious|
| F75C     | -6.495        | Deleterious|
| R71C     | -4.449        | Deleterious|
| S65R     | -1.598        | Neutral    |
| L59P     | -5.471        | Deleterious|
| T33P     | -3.672        | Deleterious|
| L13V     | -0.543        | Neutral    |
| P10A     | -0.096        | 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”.

Discussion

Insulin-like Growth Factor-1 (IGF-1) gene has been described in several researches as a candidate gene for growth. 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. This is due to the fact that nsSNPs have been reported to be linked to economically important traits and disease development. Amills et al. and Zhou et al. noted positive association between IGF-1, SNP and average daily weight in poultry. While Fang et al. 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. 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. 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. The larger the Dxy value, the greater the genetic distance while the smaller the Dxy value the closer the genetic distance between the species. This is an indication that chicken, turkey and pig are genetically distant. The evolutionary relationship of nucleotide sequences of chicken, pig and turkey

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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. 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 processes and/or similar selection pressure which the species have suffered during evolution.

**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 undergone. This new typing tool may bring insight into developing maker for IGF-1 gene.

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**Conflict of interest**

The author declares no conflict of interest.

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