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Activin Gene in Avian Species: A Case Study

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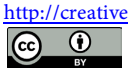

Abstract

Among the avian species, understanding the roles of activin happen to be a dominant challenge in genetic evolution due to its complexity in nature. A case study of the activin gene in avian species was carried out using bioinformatics. As a sedentary bird, guinea fowl is more susceptible to local selection processes and needs a proper genetic study for conservation. The present study provides the basis for the use of activin or its target genes for the improvement of impaired wound healing, and activin antagonists for the prevention and treatment of fibrosis and the end of malignant tumors that over-express activin. The information provided will serve as a basic tool for broader genetic diversity studies to identify valuable poultry genetic resources and major genes for the development of breeding programs. This study was done by retrieving hundred (100) nucleotides and amino acid sequences of the activin gene belonging to guinea fowl and other avians from the GeneBank, aligning the sequences using BlastP determined the percent identity and phylogenetic relationship of the activin gene of guinea fowl and other avians. The shortest activin nucleotide sequence (467 bp) was observed in chicken and the longest (39896445 bp) in duck. Using the comparative sequence analysis, it was observed that the activin gene of chickens, turkeys and guinea fowl shared percent identity ranging from 91% to 95%. The percent identity reflects the degree of relatedness of species. Although closely related (90%) in ancestral line, the activin gene of guinea fowl and quail cannot be compared with guinea fowl-turkey (95%) nor guinea fowl-chicken (90%), in both biological functions and evolutionary relationship. Finally, the percent identity and similarity in function of the activin gene of guinea fowl, turkey, and chicken were in the range of 93% - 100%, indicating that the activin gene of avians possesses similar functions, well conserved and is very effective in performing functions like increasing FSH bindings, FSH-induced aromatization, improves wound healing and enhances scar formation, regulates morphogenesis of branching

organs, and enhances ovarian folliculogenesis. The study, therefore, recommends farmers select and breed for activin genes in order to promote reproductive efficiency, thereby barricading species extinction.

Keywords

Activin, Avians, Phylogenetics, Bioinformatics

1. Introduction

The guinea fowl is a large wild bird belonging to ***Numidia*** family, related to pheasants, turkeys, and other game fowl, natively found inhabiting a variety of habitats across the African continent. Today, guinea fowl has been introduced to various countries around the world as it is farmed by humans [1]. Guinea fowls are sedentary birds, hence, more susceptible to local selection pressures than would be more mobile taxa.

Activin, a dimeric protein complex that acts in an opposing manner to inhibin to stimulate Follicle Stimulating Hormones (FSH) synthesis and secretion from the pituitary has been shown to perform various functions in livestock and avian species. In the ovarian follicle of avians, activin increases FSH binding and FSH-induced aromatization [2] [3], by participating in androgen synthesis enhancing Luteinizing Hormone (LH) action in the ovary and testis [4] [5], found activin as strongly expressed in wounded skin with its over expression in the epidermis of transgenic mice improving wound healing and enhances scar formation. Its action in wound repair and skin morphogenesis is through the stimulation of keratinocytes and stromal cells in a dose-dependent manner [6]. Activin was also found [7] as a regulator of morphogenesis of branching organs such as the prostate, lung, and especially kidney. Activin A increased the expression level of type-I collagen suggesting that activin A acts as a potent activator of fibroblasts. Lack of activin during development results in neural developmental defects [8] and its up regulation drives pluripotent stem cells into a mesoendodermal fate, and thus provides a useful tool for stem cell differentiation and organoid formation [8] [9]. Activin and inhibin are two closely related protein complexes that have almost directly opposite biological effects. Many other functions have been found to be exerted by activin, including roles in cell proliferation, differentiation, apoptosis, metabolism, homeostasis, immune response, wound repair, and endocrine function. Conversely, inhibin down regulates FSH synthesis and inhibits FSH secretion [10]. The existence of inhibin was hypothesized as early as 1916; however, it was not demonstrated to exist until Neena Schwartz and Cornelia Channing's work, after which both proteins were molecularly, characterized ten years later [11].

A mutation in the gene for the activin receptor ACVR1 results in fibrodysplasia ossificans progressiva, a fatal disease that causes muscle and soft tissue to gradually be replaced by bone tissue [2] [12]. This condition is characterized by the

formation of an extra skeleton that produces immobilization and eventually death by suffocation. This mutation causes activin A, which normally acts as an antagonist of the receptor and blocks osteogenesis (bone growth), to behave as a protagonist of the receptor and to induce hyperactive bone growth. Mutations in the activin gene have also been linked to cancer, especially diffuse intrinsic pontine glioma (DIPG) [13]. Elevated activin B levels with normal activin A levels provided a biomarker for myalgic encephalomyelitis/chronic fatigue syndrome [14]. As a causative against genetic diversity, mutations cause genetic variability within and between species.

Genetic diversity is the variation in the amount of genetic information within and among individuals of a population, an assemblage, or a community [4] [15]. Related to genetics, the most obvious is the genetic diversity between populations. Different breeds, for example, have specific genetically determined characteristics. It is possible, but very rare, that there is no genetic variation in a population [15]. This occurs in populations that are fully inbred (animals that are genetically completely identical to each other). As an umbrella term for the body of biological studies that use computer programming as part of their methodology, as well as a reference to specific analysis "pipelines" that are repeatedly used, particularly in the field of genomics [16], bioinformatics is commonly used for the identification of candidate's genes and single nucleotide polymorphisms (SNPs). Often, such identification is made with the aim of better understanding the genetic basis of disease, unique adaptations, desirable properties (especially in agricultural species), or differences between populations. In a less formal way, bioinformatics also tries to understand the organizational principles within nucleic acid and protein sequences, called proteomics. In structural biology, it aids in the simulation and modeling of Deoxyribose Nucleic Acid (DNA), Ribo Nucleic Acid(RNA), proteins as well as bimolecular interactions [16] [17].

The present study aimed at carrying out an Insilico analysis of activin gene in guinea fowl and in some avian species, thereby providing the basis for the use of activin or its downstream target gene for the improvement of impaired wound healing, and of activin antagonists for the prevention and treatment of fibrosis and of malignant tumours that over express activin. The information provided by the present study will serve as a basic tool for broader genetic diversity studies to identify valuable poultry genetic resources and major genes for the development of breeding programs. This is, because, the utilization of well-adapted poultry genetic resources is the best route to conserving them for future uses. The use of Insilico genetics analysis and bioinformatics study bridges the gap between experimental and computational biology.

2. Material and Method

2.1. Location, Retrieval of Activin Gene Sequence and Duration of the Study

The study was carried out by retrieving a hundred (100) nucleotide and amino

acid sequences of activin genes belonging to guinea fowl and other avians from the GenBank. It was done by obtaining the FAST alignment format of nucleotide and amino acid sequence of species from genes at the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/), with the use of Basic local alignment search tool (BLAST) to obtain their similar sequence. The Specific sequence of all selected species within the range of bearing ACVR2A receptor hits except for Dove whose receptor is only processed as a substrate to ACVR2B was analyzed. Shotgun Genomic analysis was processed for individual strains ranging from 700 - 900 amino acid length. The six species were selected in order of domestication and prevalence to the African Indigenous endemic specie; in addition, evolutionary trend and morphology was the criteria for limiting only to six avian species.

2.2. Experimental Procedure

Multiple sequence alignment was carried out to obtain sequence using Cluster W Software and BlastP incorporated in Molecular Evolution and Genetic Analysis software (MEGA) and National Center for Biotechnology Information respectively. Individual FASTA files were retrieved from the Genbank and queried using MEGA (Cluster W) to map out Nucleotide percentage similarity and Identity. Multiple alignment parameters used included gap open penalty scaled at 10, gap extension penalty scaled at 0.05, hydrophilic residues for proteins, hydrophilic gaps, and selected weight matrix equated to Cluster W(for DNA). Similar settings were used for the pairwise alignment except for the scoring method placed in percentile.

2.3. Multiple Sequence Alignment and Determination of Genetic Diversity

This was carried out on all the sequences using Clustal W software [18] incorporated in Molecular Evolution and Genetics Analysis Software (MEGA, version 6). The genetic diversity indices such as the number of polymorphic sites, the number of monomorphic sites, haplotype number, haplotype diversity, nucleotide substitution per site, parsimony informative site, singleton variable and conservation of activin gene of guinea fowl and other avian was determined using Bioinformatics Software.

2.4. Determination of Percent Identity

The percent identity and similarity among the amino acid sequence of the activin gene of guinea fowl and other avians were carried out by conducting a pairwise comparison of two or more sequences via the basic local alignment search tool (BLAST) incorporated in the NCBI website.

2.5. Phylogenetic Analysis

The phylogenetic relationship among the activin gene of guinea fowl and other avian was determined using the Molecular Evolution and Genetic Analysis (MEGA)

software. A phylogenetic tree was constructed using the Neighbor Joining statistical method, to determine the evolutionary relationship of activin gene sequence among avian species.

3. Result and Discussion

Retrieval of Nucleotide and Amino Acid Sequences of *Activin* Gene

The length of the nucleotide sequence of the *ACTIVIN* gene varied from 467 base pairs to 39896445 base pairs (**see supplementary information**) in species although similar protein templates. 100 sequences (Figure 1) for various species of phylum were retrieved from NCBI. Gene cutting across 773 aa (Amino acids) for all six species was selected and probed for sequence similarity/identity (Figure 2), motifs, putative domain construct (Figure 3), and genome computational analysis (Figure 4) encoding bit of similar homology across strains. The gene sequence for chicken, turkey, guinea fowl, quail and duck *ACTIVIN* gene contained; 467, 38723565, 4861, 2496, and 39896445 base pairs respectively. The shortest *ACTIVIN* nucleotide sequence (467 base pairs) was observed in chicken while the longest *ACTIVIN* nucleotide sequence (39896445 base pairs) was observed in duck.

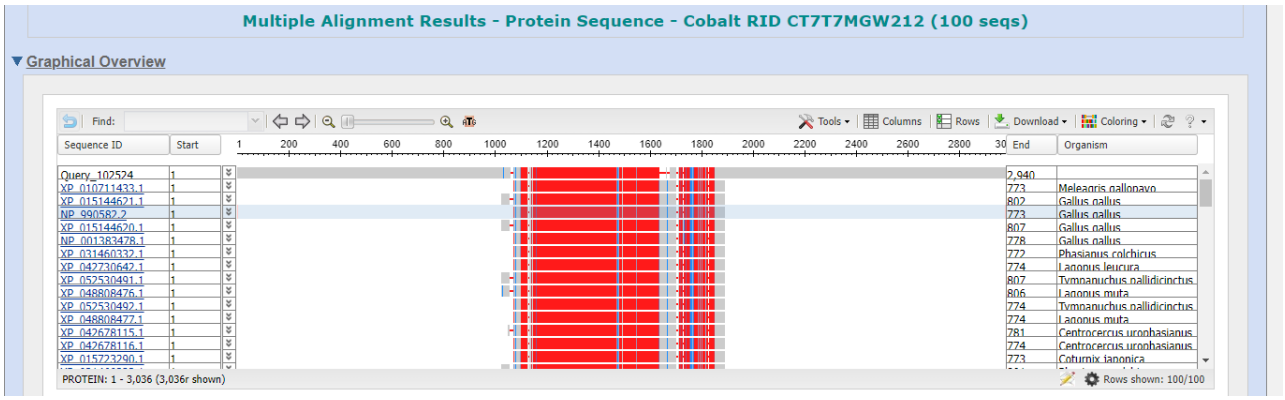

Figure 1. 100 sequences randomly chosen across target species.

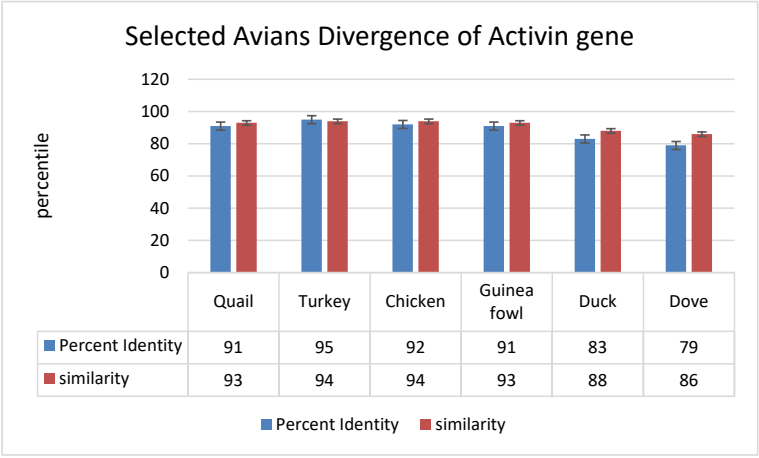

Figure 2. Percentage identity/similarity of activin gene among avian species.

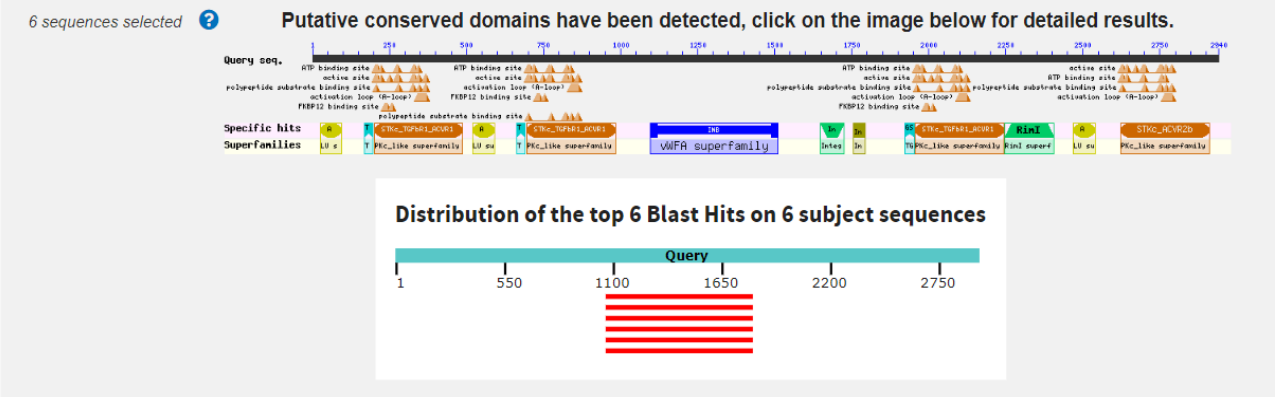

Figure 3. Distribution of domain hits showing similarity in the six specie sequences selected.

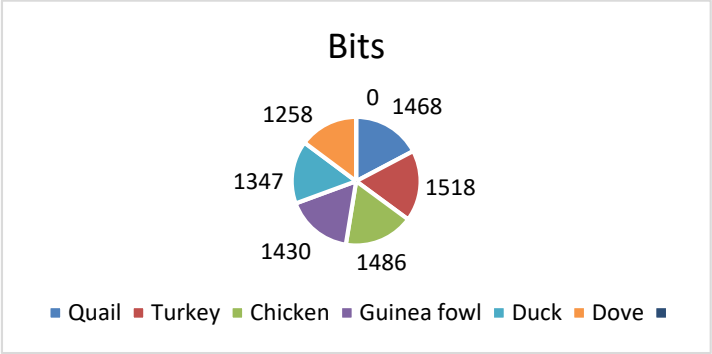

Figure 4. Comparative genomic bits.

It can be observed that morphology is directly related to an increase in nucleotide sequence length as the duck has the highest nucleotide sequence length followed by turkey (**Figure 5**) and quail respectively while chicken has the least. ACVR2b; STKs catalyze the transfer of the gamma-phosphoryl group from ATP to serine/threonine residues on protein substrates. ACVR2b (or ActRIIB) belongs to a group of receptors for the TGFbeta family of secreted signaling molecules that includes TGFbeta, bone morphogenetic proteins (BMPs), activins, growth and differentiation factors (GDFs), and anti-Mullerian hormone, among others. ACVR2b is one of two ACVR2 receptors found in vertebrates. Type II receptors are high-affinity receptors which bind ligands, auto phosphorylate, as well as trans-phosphorylate and activate low-affinity type I receptors. ACVR2 acts primarily as the receptors for activins, nodal, myostatin, GDF11, and a subset of BMPs. ACVR2 signaling impacts many cellular and physiological processes including reproductive and gonadal functions, myogenesis, bone remodeling and tooth development, kidney organogenesis, apoptosis, fibrosis, inflammation, and neurogenesis [19] [20].

The ACVR2b subfamily is part of a larger superfamily that includes the catalytic domains of other STKs (refer to **Figure 6**), protein tyrosine kinases, RIO kinases, aminoglycoside phosphotransferase, choline kinase, and phosphoinositide 3-kinase.

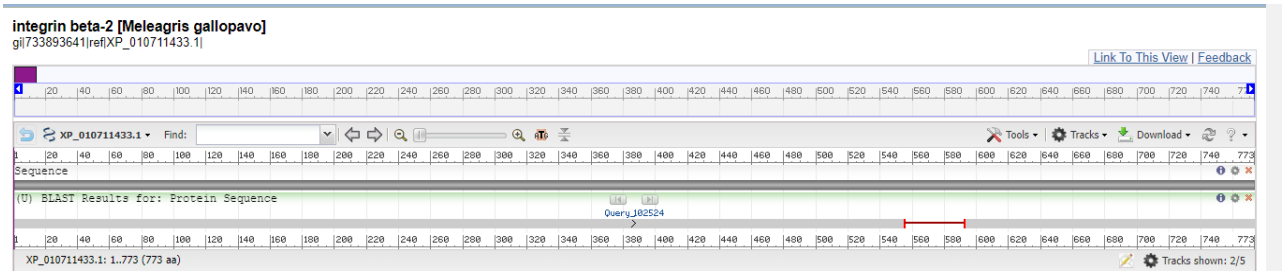

Figure 5. Typical Blasted Result of one of the target specie (Turkey).

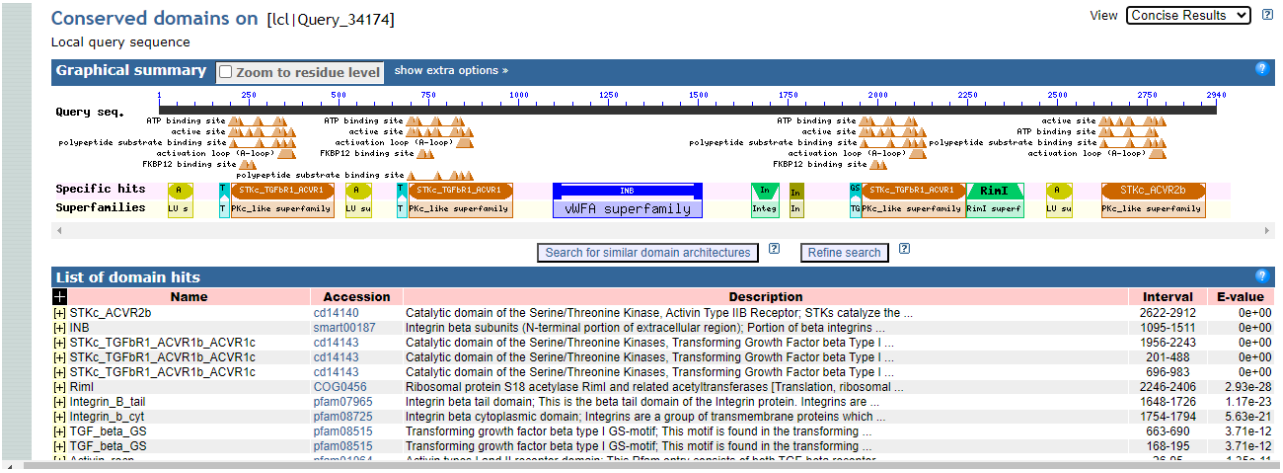

Figure 6. List of domain hits with E-value for activin and transforming beta growth factor ranging from 0 - 3.7.

Sequences within the range of 773 Amino acids were chosen and analyzed independently for the species. This ascertain supports the evidence that the activin gene is limited in avians cytoskeleton with the extracellular matrix and they transmit signals bidirectionally between the extracellular matrix [21] and cytoplasmic domains and vary in direct proportion to morphological index. Integrins link the actin. The nucleotide collection consists of GenBank + EMBL + DDBJ + PDB + RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100 Mb. The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry (NCBI).

100 sequences (**Figure 1**) were randomly chosen across the target species: Meleagris gallopavo, Numida meleagris, Courtnix courtnix, Anas platyrhynchos, Columba livia and Gallus gallus. Beta-integrins are primarily responsible for targeting integrin dimers to the appropriate subcellular locations. Activin genes have been reported to play various roles in a dynamic matrix; The functions of activins through activin receptors are pleiotropic, cell type-specific and contextual, and they are involved in etiology [22] [23] [24].

These receptors contain an extracellular domain that binds ligands, a single transmembrane region, and a cytoplasmic catalytic kinase domain (**Figure 3**).

The related level of reduction in Integrin beta 2 is observable across species in

the evolutionary trend (Figure 7). The negligible amount of the gene found in the predicted low quality of the activin gene hierarchy suggests that the avians have a functional biological pathway for regulating the biosynthesis of the activin gene. The percent identity of chicken, turkey, and guinea fowl *ACTIVIN* gene and other avian species were obtained using comparative sequence analysis. It was revealed that the activin gene of chicken, turkey and guinea fowl shared percent identity ranging from 91% to 95%. The tree was produced using BLAST pairwise alignment on NCBI.

Molecular pathways (Figure 8) in the transmission of activin signals lead to actin reorganization and epithelial cell migration. Activin increases the number of synaptic contacts and the length of dendritic spine necks by modulating spinal actin dynamics [25].

It was obtained that guinea fowl and quail shared 91% identity, while turkey, dove, duck and chicken shared 95%, 79%, 83%, and 92% identity respectively. Percent identity refers to a quantitative measurement of the similarity between two sequences (DNA, amino acid or otherwise). Closely related species are expected to have a higher percent identity for a given sequence than would more distantly related species, and thus percent identity to a degree reflects relatedness. Guinea fowl and quail shared 91% identity of activin gene; an indication that the evolutionary relationship between guinea fowl and quail is moderately high. However, chicken shared 92% identity which is 1% higher than that of guinea fowl and quail relationship and is therefore higher in evolutionary relationship than the former and can perform the same biological function (promoting follicular dominance) by recruiting folliculogenesis during each ovarian cycle [26]. This further buttresses that they come from the same ancestral line. Nevertheless, turkey is highest in the percent identity of activin gene which implies the relatedness in the evolutionary tree and the gene can perform same biological function (increasing fertility potential) in both species by breaking down germ cell nest [27]. Guinea fowl and quail share percent identity of activin gene 91% although closely related in ancestral line but the relatedness can't be compared with that of turkey: chicken in both biological function and evolutionary relationship.

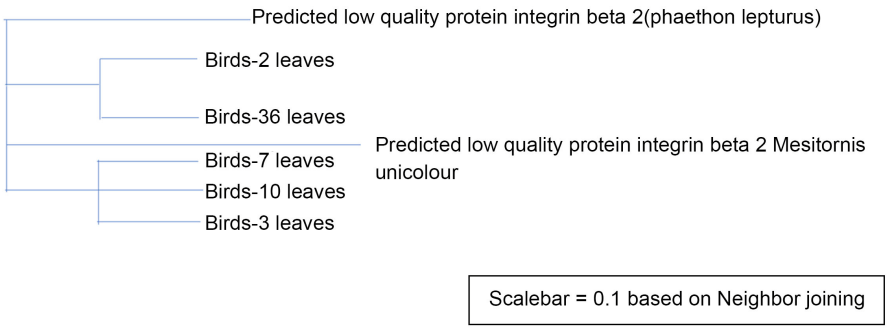

Figure 7. Predicted low quality of activin gene in hierarchy of evolutionary trend.

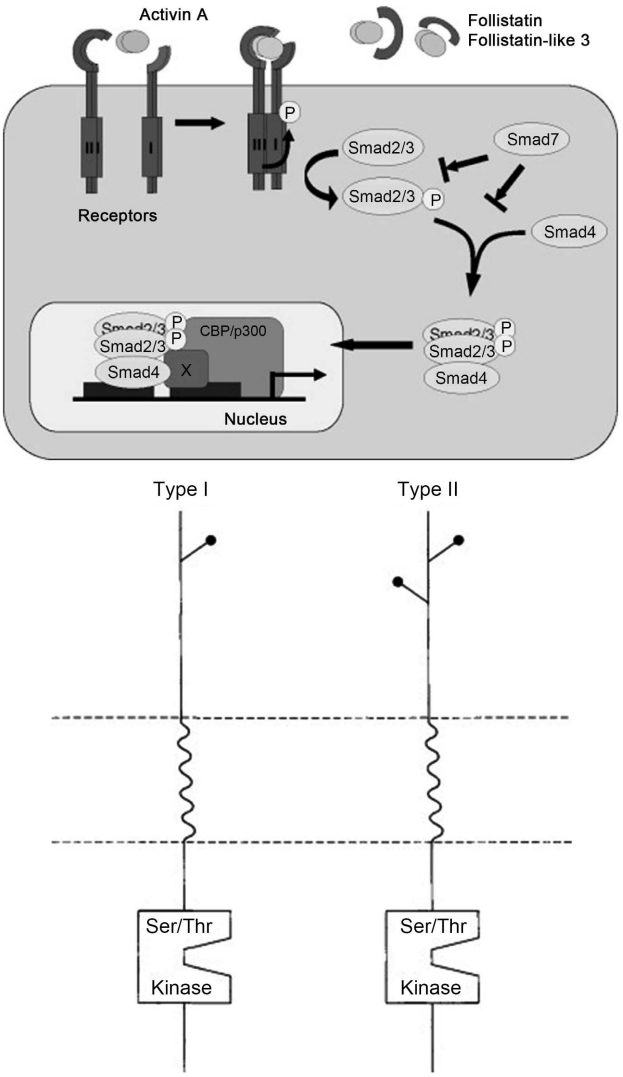

Figure 8. Mechanism of action of activin gene (Source: Peter C.K. Leung, Chun Peng, in Encyclopedia of Hormones, 2003).

Guinea fowls share a percent identity of 91% with quail, closely related in ancestral line more than duck and dove which share the identity of 83% and 79% respectively. They all share activin function similarly in reducing the sensitivity of aging animals to the effect of dietary restriction. Closely related species share a significant same value of Percent identity which translates to the protein levels, nucleotide sequence as well as mRNA in the organism's profile. This gives further insights into how the evolutionary track record has acquired stability in fostering genes required for maintenance and growth. Conservation of genes results in similar observable phenotypes which help species that share the same degree of relatedness thrive.

Percent similarity is homology among proteins or DNA which is typically inferred from their sequence similarity [26]. From the above result, chicken has a percent similarity of 95% with turkey and guinea fowl which means they are more closely related in the evolutionary role and biological function (promoting

oligodendrocyte development and CNS myelination) of activin gene in both species [24] [26]. Whereas chicken and turkey have the similarity of 100% which is certainly higher than that of Quail and guinea fowl, this implies that chicken and turkey are from the most common recent ancestor closely related in both function and specificity (regulating morphogenesis of branching organs such as lungs and most especially kidney) [20] [26].

Chicken shares the similarity of 86% with both duck and dove, which is certainly lower in the ancestral relationship than that of chicken with turkey and guinea fowl. Quail and guinea fowls have a similarity of 100% which is certainly higher than that of duck and dove. This implies that Quail and guinea fowl are from the most common recent ancestors closely related in both function and specificity than other species (regulating proliferation and adhesion of hemocytes) NCBI links: precomputed BLAST. Guinea fowl shared a similarity of 93% with quail higher than that of duck and dove. Summarily, chicken shared 100% similarity with turkey and quail shares 100% similarity with guinea fowl while dove shared 86 % similarity with duck. Percent similarity, also called sequence similarity checks sameness in Amino acids in addition to Similar residues. These residues can be checked based on chemical composition or structures.

The evolutionary trend has over the years been maintained through various phenomenon, pathways, molecular interaction as well as genetic conservation. Genetic conversation hinges on the degree of sequence identity and similarity shared amongst species of the same ancestral chord, phylum, and trends. The analysis shows how avian has conserved similar functions in genes as well as sharing a direct relationship in morphology. This will help geneticists make proper informed decisions.

The independent treatment type is statistically different with P value= 0.02. The divergence of the activin gene though not quantitatively discrete but the relative value suggests a closeness in the taxonomy (**Figure 2**) as to the specific function and role which this gene plays while delimiting excessive transcription.

The gene cuts across the targeted species with varying lengths in homology which is not a function of hierarchical order in taxonomy (**Figure 4**).

The bits are a function of the comparative genomics derivative of each species. The basic concept of sequence homology showed that turkey, chicken, and quail share similarities in homology to other species. This contradicts of the taxonomy as the hierarchy in taxonomy doesn't correlate with the sequence homology [28]. Although dove and duck shared the least sequence homology, duck has wider genomic coverage encoding activin gene in the highest base pair of39896445 such is observable too as seen in turkey, chicken and Guinea fowl. (**Table 1**)

The phylogenetic tree in (**Figure 9**) showed the evolutional relationship between guinea fowl, turkey, chicken, and quail. The above shows the evolutionary relationship of avian activin gene as they shared various percent identity and similarity showing the ancestral relatedness and similarity in biological functions respectively [29]. This equally gives the breeder an insight into the various functions and how diversely activin gene can be used to improve each species.

Table 1. Retrieved nucleotide and amino acid sequences of the activine gene of the selected species with their accession numbers and sequence length.

Species	n	Genbank accession number	Location	Nucleotide sequence length	Amino acid length
Chicken	4	NC_006094.5	Chromosome 7	467 bp	803
Turkey	2	NC_015017.2	Chromosome 7	38,723,565 bp	773
Guinea fowl	2	NC_034413.1	Chromosome 5	4881 bp	773

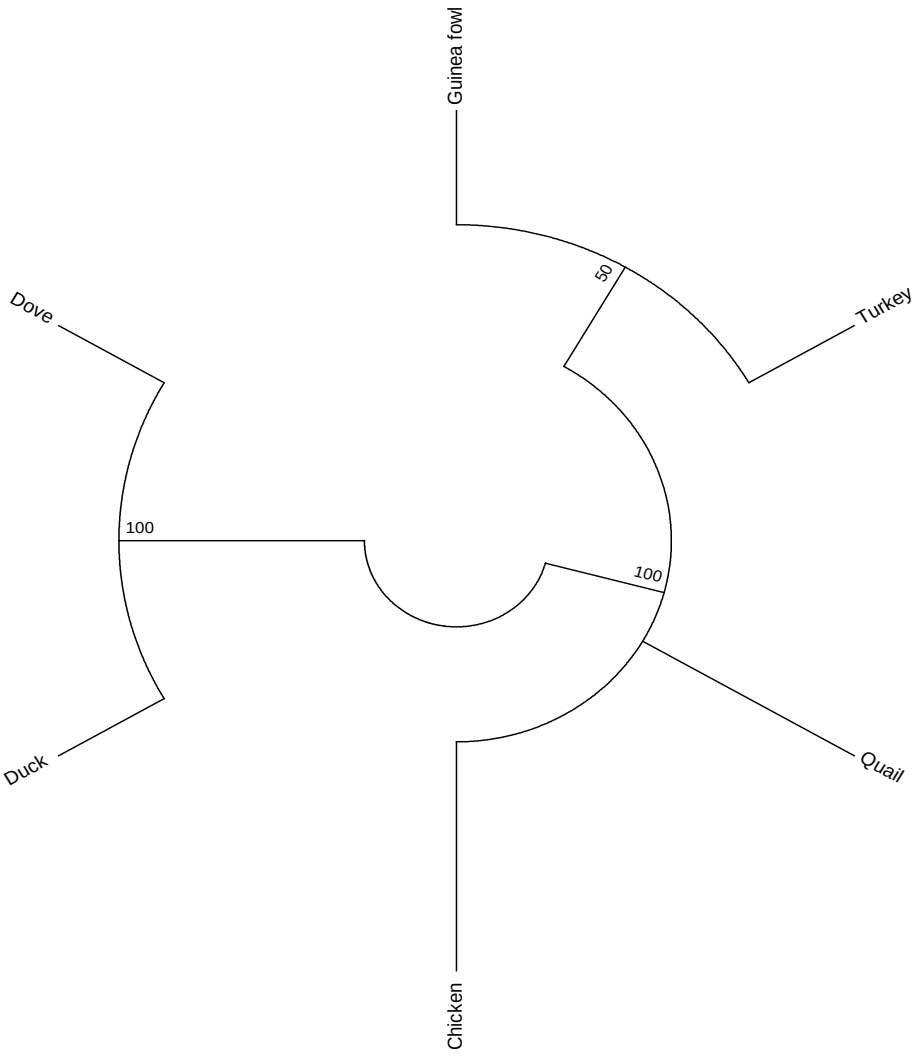

Figure 9. A phylogram showing the evolutionary relationship of avian activin gene.

Phylogenetic analysis can be used to discover gene preservation events which are conservation between modern and classic methods. Modern phylogeny shows genetic material (which is DNA and protein sequence) that frequently cause them to group together rather than classic method that deals with physical or morphological features. The avian species had a common ancestor because they were on the same phylogenic tree branch which confirms the morphological classification of chicken, turkey, and guinea fowl as Aves (non-ruminant) animals and

agrees with the taxonomy of National center for biotechnology information (NCBI) even though some are more closely related than others. Chicken and quail are classified under the same clade (group of animals or organisms derived from a common ancestor species), they share most recent common ancestor, more closely related and highly similar than others because the distance between the length of the clades are closer compared to other avians in the phylogenetic tree. Although chicken and turkey are in the same clade, but they did not share most recent common ancestor because the distance between the length of the clade is high compared to that of the chicken and quail; this can be because of changes that might occur in evolution or little changes in the coding sequence of activin gene. Chicken and turkey showed similarities but did not share most recent common ancestor. Guinea fowl is more closely related to turkey than chicken and quail but closely related to chicken than quail because they have the highest clade distance in the phylogenetic tree. Evolutionary trees show how two or more organisms are derived or come to existence and not how similar they are in function [28]. This result shows that avian activin genes can adapt to similar environmental conditions and can have close external resemblance. The necessity of the gene has shown through evolutionary trend how species similarity and identity has fostered such translation thus inhibiting extinction. Evolutionary relationship analysis helps the breeders to understand how species of activin gene in avians are related to use the knowledge to identify species that can give the same phenotype. Since their genes are closely related, it also enables farmers to identify individuals or populations of animals that can adapt to similar environmental conditions.

Genetic diversity is useful especially for selecting animals with high reproductive efficiency and fertility potential which are most preferred by ranchers. Diversity in activin gene may lead to variation in the expression of function with avians having more of such variation.

Egom *et al.* [30] reported that diversity in avian IGF-1 (insulin growth factor-1) gene gave rise to variation in the gene, which is a useful tool in evolution and natural selection. Therefore, it can be said that chicken activin gene has high adaptability and can survive the changing environmental condition since diversity helps ensure survival of species. Thus, although these species are closely related from their phylogeny, activin gene will perform more biological function in chicken than in other avian species studied. The higher the base pair alignment the higher the polymorphism and the higher the gene mutation. In breeding and selection of activin gene for ovarian folliculogenesis and increased fertility potential, the mutant gene is preferable, hence activin gene will probably have higher level of mutant genes.

4. Conclusion and Recommendation

From the result obtained, the percent identity and similarity in function of the activin gene of guinea fowl, turkey, and chicken were in the range of 93 - 100

percent, indicating that the activin gene of avian possesses similar functions, well conserved and are very effective in performing functions like increasing FSH binding, FSH-induced aromatization, improves wound healing and enhances scar formation, regulates morphogenesis of branching organs, and enhances ovarian folliculogenesis. The phylogenetic relationship of the activin gene in the six species studied supports the taxonomy classification of NCBI. The study also showed that the activin gene of chicken, turkey, and guinea fowl also originated from a common ancestor with turkey and guinea fowl sharing a most recent common ancestor. From this study, it can be recommended that since the effect of the activin gene in avian promotes ovarian folliculogenesis that enhances reproduction; farmers can select and breed for the activin gene in order to promote reproductive efficiency thereby barricading species extinction. Activin gene sequences available in the Genbank were from exotic breeds. It is necessary to carry out research on local breeds of chicken, guinea fowl and turkey so that their sequence can also be available in the Genbank for comparative analysis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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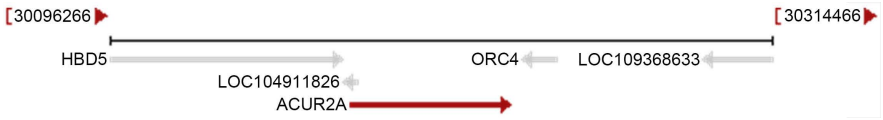
Supplementary

Table S1. ACVR2A ortholog spans across ORC4 and MBD5.

Location: chromosome: 7
Exon count: 12



Annotation release	Status	Assembly	Chr	Location
103	Current	Turkey_5.1 (GCF_000146605.3)	7	NC_015017.2 (30175339…30227988)
Build 1.1	Previous assembly	Turkey_2.01 (GCF_000146605.1)	7	NC_015017.1 (37322572…37378077)

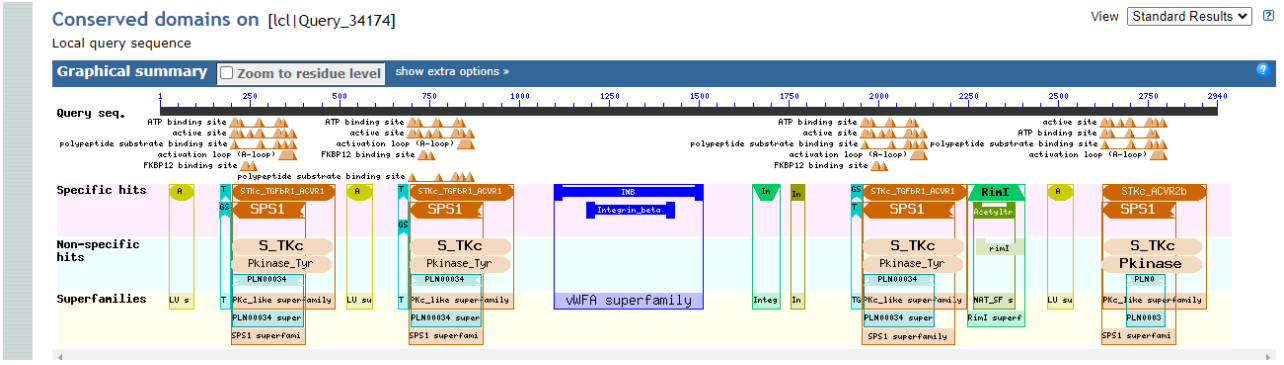


Figure S1. Conserved domain hits in activin orthologs.