



EFFECTS OF MUTATIONS IN FOLLICLE STIMULATING HORMONE RECEPTOR (FSHR) GENE IN SELECTED POULTRY SPECIES USING IN-SILICO METHODS

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ABSTRACT

The study investigated the effects of mutations in the Follicle Stimulating Hormone Receptor (FSHR) gene in some selected poultry species, specifically chickens (*Gallus gallus*), ducks (*Anas platyrhynchos*), and geese (*Anser cygnoides*), utilizing *in-silico* methods. The FSHR gene sequences were retrieved from National Center for Biotechnology Information (NCBI), revealing varying base pair lengths: 3,516 in chickens, 3,508 in ducks, and 2,652 in geese. An analysis of evolutionary divergence demonstrated varying degrees of genetic distance, with ducks and geese exhibiting the closest genetic similarity (0.038), while chickens and geese displayed the greatest divergence (0.958). *In-silico* characterization of the FSHR gene identified 27 genetic variants, with Sorting Intolerant from Tolerant (SIFT) scores assessing the potential impact of these mutations. Among these, 13 variants were predicted to be deleterious, likely affecting the gene's function significantly and 14 were beneficial. These findings offer valuable insights into the genetic variability and possible adaptive differences in FSHR gene function among poultry species. In conclusion, the study highlights significant genetic divergence in the FSHR gene among chickens, ducks, and geese, with the closest evolutionary relationship observed between ducks and geese. Exploring the potential of using FSHR gene variants as markers in selective breeding programs to improve reproductive efficiency and overall poultry production is recommended.

Keywords: Mutation, Follicle, Hormone, Gene, Stimulants

INTRODUCTION

The poultry industry has significantly expanded since 2000, now ranking second to pork in global meat production (USDA, 2014). Poultry is increasingly popular due to its health benefits, including lower triglycerides, cholesterol, and saturated fats compared to red meat (Hoffman & Falvo, 2022; Drouin-Chartier & Desroches, 2024). Additionally, poultry is affordable, available in convenient portions, and free from most religious dietary restrictions (Kumar & Azzam, 2024; Falkenberg & Larson, 2022). Egg production, a polygenic trait with low to moderate heritability, is influenced by endocrine and environmental factors such as photoperiod length and feeding allowances (Garcia & Gonzalez, 2023; Fassenko & Hocking, 2022). The goal of modern poultry breeding is to enhance egg production while reducing broodiness, with the process controlled by the hypothalamic-pituitary-gonadal (HPG) axis (Gonzalez & Lee, 2023). The Follicle Stimulating Hormone Receptor (FSHR) plays a vital role in reproductive physiology by mediating the effects of follicle-stimulating hormone (FSH), which regulates gonadal development (Miller, 2022). Mutations in the FSHR gene can impact reproductive efficiency, making it a key focus of genetic research (Sang & Ahn, 2022). By studying these mutations computationally, insights into their biological effects and



applications in poultry breeding can be gained (Huang *et al.*, 2022; Li *et al.*, 2023). FSHR is primarily expressed in the gonads, where it is essential for follicle maturation and ovulation (Rood & Szkudlinski, 2023). Mutations can disrupt FSHR function, leading to reproductive disorders and reduced productivity (Zhao *et al.*, 2022). These genetic alterations can include point mutations, insertions, deletions, and duplications, affecting receptor function and signaling pathways (Gong *et al.*, 2023). In silico methods such as homology modeling and molecular dynamics simulations help visualize how mutations affect FSHR structure and function (Wang *et al.*, 2023). Algorithms like SIFT evaluate the impact of mutations on protein function (Kumar *et al.*, 2023). Different poultry species exhibit variability in FSHR gene sequences, and identifying species-specific mutations can enhance reproductive efficiency through genetic interventions (Liu *et al.*, 2022). The objective of this study is to explore the effects of mutations in Follicle Stimulating Hormone Receptor (FSHR) gene in selected poultry species using in silico methods.

MATERIALS AND METHODS

Retrieval of Follicle Stimulating Hormone Receptor (FSHR) Gene of Poultry

Six sequences of the Follicle Stimulating hormone receptor (FSHR) gene were analyzed, comprising two sequences from each of chicken, duck and geese retrieved from the National Center of Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov). With a total of six nucleotide sequences, two nucleotide sequences were carefully selected, each with the following Gen bank accession numbers; NM205079.2, XM046913182.1 for chicken; XM021267215.3, XM021267214.3 for duck; XM013192472.2, XM048078417.1 for geese.

Sequence Alignment and Translation

Sequences were aligned, translated, and compared using ClustalW, following the methodology outlined by Larkin *et al.* (2007). The International Union of Biochemistry (IUB) substitution matrix was utilized, along with a gap open penalty of 15 and a gap extension penalty of 6.66. These alignments were employed to assess genetic distance and establish evolutionary relationships among the sequences.

Identification of Variations in Follicle Stimulating Hormone Receptor (FSHR) Gene Sequences

To identify variants of the (FSHR) gene, the Basic Local Alignment Search Tool (BLASTx) available on the NCBI BLAST website (www.ncbi.nlm.nih.gov) was utilized. The (FSHR) gene sequence was copied into the "Enter query sequence" field, and the search was conducted against the standard non-redundant protein sequence database using default parameters. The BLASTx algorithm was employed to compare the query sequence with entries in the selected database. Pairwise alignments with dot plots were chosen to pinpoint mutations and similarities between the query sequence and its matches. Sequence similarity search tools like BLAST (Jang *et al.*, 2020) are widely used for querying sequence databases to identify similarities with known sequences.

Characterization of variations in Follicle Stimulating Hormone Receptor (FSHR) gene sequences.

To characterize gene variants, the SIFT (Sorting Intolerant from Tolerant) website (<https://sift.bii.a-star.edu.sg>) was utilized. The SIFT sequence protein tool was selected to predict the impact of amino acid substitutions on protein function. The protein sequence was entered into the SIFT input field, where the appropriate database and parameters were chosen. The SIFT algorithm was then applied to forecast the effects of these substitutions on protein functionality. A significance threshold of 0.05 was applied. Substitutions scoring below 0.05



were predicted as deleterious, while those scoring above 0.05 were predicted as tolerant. SIFT utilizes UniProt for retrieving protein sequences and annotations, yet both serve distinct roles in bioinformatics. Developed by Ng and Henikoff in 2001, SIFT is widely employed for predicting the impact of amino acid substitutions on protein function. In silico functional analysis of non-synonymous mutations was conducted using SIFT (Thomas *et al.*, 2003).

Evolutionary Relationship

The sequences obtained were aligned using Clustal W within Mega, followed by calculation of genetic distances. Unweighted Pair Group Method with Arithmetic Mean (UPGMA), trees were then constructed using the P-distance model, employing pairwise deletion for handling gap and missing data. These trees were utilized to assess the evolutionary relationships among the selected poultry species (chicken, duck and geese) based on genetic divergence. Bootstrap confidence values (Tuffley and steel 2022; Zhang and Zhang 2023,) were computed to evaluate the reliability of the trees, with 1000 iterations conducted using MEGA 5.1 software (Tamura *et al.*, 2011).

RESULTS AND DISCUSSION

Retrieved Nucleotide Sequences and Base Pairs

The variation in sequence length in base pairs (bp) of the FSHR gene between species ranged between 2471 bp and 3516 bp (Table 1). All these sequence lengths partial coding sequences (partial CDS) because they had less than six thousand base pairs (<6000 bp) each.

Table 1: Accession Number and Base Pairs of chicken, duck and geese FSHR gene

Specie	Accession Number	Base Pair
<i>Gallus gallus</i> (Chicken)	NM205079.2	3,516
	XM046913182.1	2,947
<i>Anas platyrhynchos</i> (Duck)	XM021267215.3	3,508
	XM021267214.3	3,489
<i>Anser cygnoides</i> (Geese)	XM013192472.2	2,471
	XM048078417.1	2,652

Alignment, Translation, and Comparative Analysis of Sequences

The chicken, duck, and geese FSHR gene sequences were aligned, translated, and compared using Clustal W, following the methodology detailed by Larkin *et al.* (2007). This process utilized an IUB substitution matrix with a gap open penalty set at 15 and a gap extension penalty of 6.66. Analysis of the predicted amino acid sequences revealed different levels of substitutions among the species under investigation. Periods (.) indicate identical amino acids, numbers on the right side denote site numbers, question marks (?) denote missing amino acids, and asterisks (*) signify indels, as depicted in Figure (1). Multiple sequence alignments (MSA) are important in biology for comparing similar sequences to understand evolutionary relationships, analyze protein structures, and predict protein functions.

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#JQ711180.1_Gallus_A KI*VYMLLLI KFLIFRMS? - ----- [169]
#AB076403.1_Gallus_B ...-QTTFQAQ ----- [169]
#EF069435.1_Anas_A ...-KTTFAQ GSSTPGTD*S VRCPER*QQR RLLGRR*LP [169]
#EF069434.1_Anas_B ...-KTTFAQ GSSTPGTD*S VRCPER*QQR RLLGRR*LP [169]
#KY828429.1_AnserA ----- [169]
#AY032689.1_AnserB_SRC-PVSHPM KTL-LTLL----- [169]
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Figure 1: Multiple Sequence Alignment of the Reference Amino Acids of Chicken, Duck and Geese FSHR gene Using ClustalW



Genetic Distances

The estimated distance matrix for follicle stimulating hormone receptor gene between six sequences considered showed a minimum distance of 0.038 between duck and geese, while the maximum distance is 0.976 between duck and chicken (Table 2).

Table 2: Estimates of Evolutionary Divergence between Sequences of Chicken, Duck and Geese FSHR gene

	Chicken1	Geese 2	Chicken 2	Duck1	Duck2	Geese1
Chicken 1						
Geese 2	0.169					
Chicken 2	0.872	0.958				
Duck 1	0.170	0.038	0.923			
Duck 2	0.190	0.068	0.976	0.028		
Geese1	0.698	0.000	0.958	0.038	0.068	

The genetic distance provided insights into the relationship among the three selected poultry species. The analysis indicated that ducks and geese were closely related, with a genetic distance of 0.038, suggesting a shared ancestry as depicted in figure 5 and 6 of the phylogenetic tree. This is in line with the evaluation of the research of Basha *et al.*, (2016), which documented same. Similarly, Livezey and Zusi, (2007), found that ducks (Anatidae) and geese (Anserinae) are indeed closely related, but they are placed in separate sub families within the family of Anatidae. It also indicated that ducks and chickens had a maximum distance of 0.976. This is similar to the report of Basha, who indicated that duck and chicken have the farthest distance (Basha *et al.*, 2016). Also, the reports of Chen *et al.* (2014) and Jarvis *et al.* (2014) which stated that ducks and chicken are relatively distantly related. Genetic distance is the degree of genetic variation or difference between organisms or populations, often measured by comparing their DNA sequences or genetic markers (Scott and Jon, 2021; Hartl and Clark, 2006), Genetic distance is a measure of the genetic divergence between species. The genetic distance measure chosen depends on how it will be applied. These measures can be utilized for population classification or for conducting evolutionary comparisons (Liu and Zhang, 2023; Santos and Navarro, 2024; Zhang *et al.*, 2023).

Evolutionary Relationship

The UMPGA consensus tree in figure 2 and 3 shows that all the species had a common ancestor. However, the evolutionary relationship between the species showed that duck and geese have a common ancestor. The evolutionary relationship in Figures 2 and 3 indicated that species coming from the same nodes had a common ancestor. The sequences considered for the follicle stimulating hormone receptor (FSHR) gene showed that seven nodes stemmed from the root. Chicken 1 and Chicken 2, Duck 1 and Duck 2, and Geese 1 and Geese 2 all had a common ancestor. However, evolutionary relationship between the species showed that duck and geese have a common ancestor which is similar to the report of Islam *et al.* (2014); Kim *et al.*, 2022; Miller and Rhymer, (2021) and Lu *et al.* (2015), which all documented that duck and geese shared a common ancestor. Similarly, according to the observation of Basha *et al.*, (2016), it dendogram described a closely genetic relationship between duck and geese. The identified genetic variation in the FSHR gene within the studied populations played a pivotal role in driving evolutionary adaptation. This variation has provided the genetic diversity necessary for natural selection to act upon, allowing beneficial alleles to increase in frequency within the population. Additionally, it has enabled individuals

to adapt to their environments effectively, thereby ensuring the survival and resilience of the population as a whole.

Phylogenetic trees illustrate evolutionary pathways, comparing ancestral nucleotide and amino acid sequences among species. Each tree provides valuable insights into evolutionary relationships (Bibinus *et al.* 2016). The phylogenetic tree showed species-specific clustering with some overlap between species, suggesting trans-species evolution possibly influenced by sequence coding. UPGMA consensus trees based on nucleotide and amino acid sequences indicated that ducks, geese, and chickens shared a common ancestor, with ducks and geese being the most closely related.

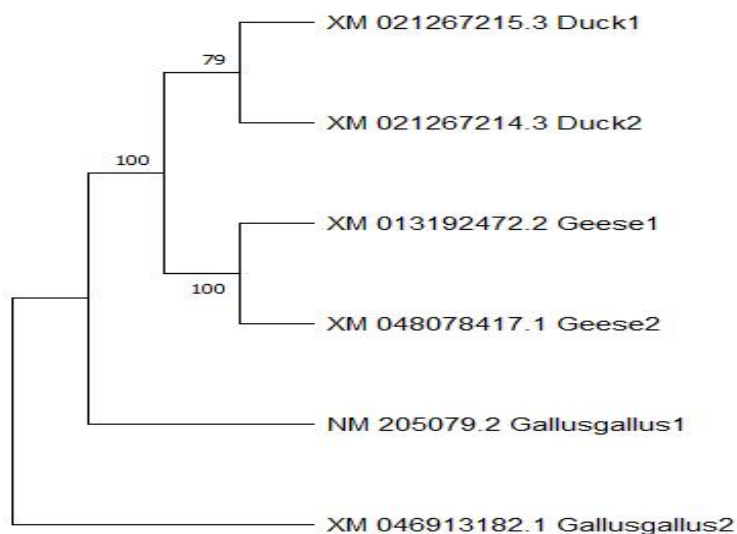


Figure 2: Showing the Phylogenic Relationship of Chicken, Duck, and Geese

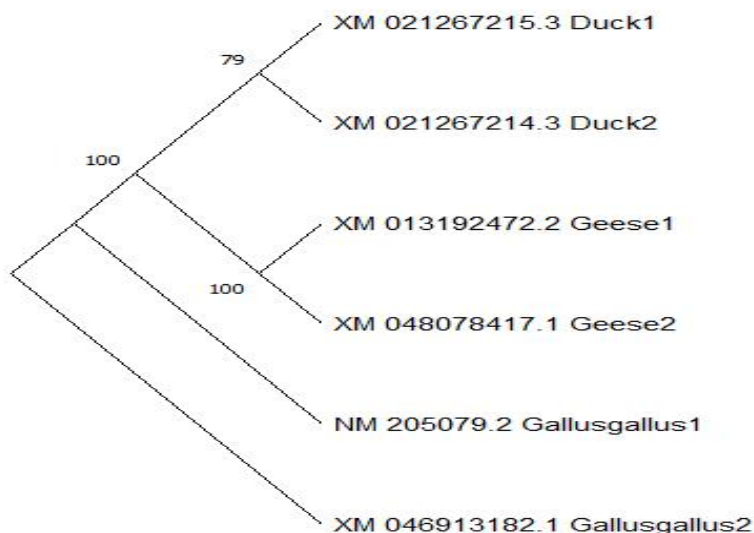


Figure 3: Showing the Phylogenic Relationship of Chicken, Duck, and Geese

Characterization of the Sequences Considered

There were Twenty-seven (27) substitutions of FSHR gene of the sequences considered. Fourteen (14) were tolerant, namely; Alanine 88 Glycine, Glycine 4 Aspartic Acid, Threonine 22 Methionine, Leucine 24 Arginine, Glutamic Acid 26 Alanine, Leucine 24 Histidine, Isoleucine 35 Serine, Serine 13 Cysteine, Histidine 32 Arginine, Leucine 35



Histidine, Glutamic 37 Alanine, Threonine 46 Serine, Phenylalanine 13 Serine, Serine 25 Leucine. Thirteen (13) substitutions namely; Glycine 17 Cysteine, Lysine 9 Glutamic Acid, Threonine 10 Arginine, Leucine 23 Histidine, Glutamic Acid 3 Aspartic Acid, Alanine 6 Threonine, Serine 29 Isoleucine, Glutamic Acid 2 Arginine, Aspartic Acid 3 Glutamic, Lysine 4 Threonine, Cysteine 14 Proline, Threonine 10 Isoleucine, Valine 15 Glycine were deleterious.

Table 3: Characterization of Follicle Stimulating Hormone Receptor (FSHR) Gene of Chicken, Duck and Geese

S/N	Variants	Sift Score	Effects
1	Alanine 88 Glycine	0.08	Tolerated
2	Glycine 4 Aspartic Acid	0.10	Tolerated
3	Glycine 17 Cysteine	0.01	Deleterious
4	Threonine 22 Methionine	0.12	Tolerated
5	Leucine 24 Arginine	1.00	Tolerated
6	Glutamic Acid 26 Alanine	0.52	Tolerated
7	Leucine 24 Histidine	1.00	Tolerated
8	Isoleucine 35 Serine	0.16	Tolerated
9	Lysine 9 Glutamic Acid	0.00	Deleterious
10	Threonine 10 Arginine	0.12	Deleterious
11	Serine 13 Cysteine	0.12	Tolerated
12	Leucine 23 Histidine	0.00	Deleterious
13	Glutamic Acid 3 Aspartic Acid	0.00	Deleterious
14	Histidine 32 Arginine	0.87	Tolerated
15	Leucine 35 Histidine	1.00	Tolerated
16	Glutamic 37 Alanine	0.60	Tolerated
17	Threonine 46 Serine	0.78	Tolerated
18	Alanine 6 Threonine	0.00	Deleterious
19	Phenylalanine 13 Serine	0.74	Tolerated
20	Serine 25 Leucine	0.23	Tolerated
21	Serine 29 Isoleucine	0.00	Deleterious
22	Glutamic Acid 2 Arginine	0.00	Deleterious
23	Aspartic Acid 3 Glutamic	0.00	Deleterious
24	Lysine 4 Threonine	0.00	Deleterious
25	Cysteine 14 Proline	0.02	Deleterious
26	Threonine 10 Isoleucine	0.00	Deleterious
27	Valine 15 Glycine	0.02	Deleterious

SIFT evaluates the effects of amino acid substitutions based on the conservation of residues within a protein family (Kumar *et al.*, 2023). SIFT, which stands for "Sorting Intolerant from Tolerant," is a bioinformatics tool used to predict the potential impact of amino acid substitutions on protein function (Kumar *et al.*, 2021). It assesses whether a particular amino acid change in a protein sequence is likely to be tolerated or deleterious by analyzing the conservation of amino acid residues across related proteins or species (Jang *et al.*, 2023). It assumes that conserved amino acids are critical for protein function, so alterations at these positions are often predicted to be detrimental. For example, if a position in a protein alignment is consistently occupied by glutamic acid, a substitution with a different amino acid is likely to disrupt protein function. Conversely, if a position predominantly features hydrophobic amino acids such as alanine, proline, or tryptophan, SIFT typically predicts that substitutions with other hydrophobic residues will be well-



tolerated. In contrast, changes to residues with distinct properties, like charged or polar amino acids, are generally anticipated to negatively affect protein function (Kumar *et al.*, 2021). To predict the effect of an amino acid substitution on protein function, SIFT evaluates both the position of the change and the nature of the substitution. It achieves this by aligning the query protein sequence with related proteins and assessing which amino acids are prevalent at each position in the alignment. SIFT then infers that substitutions involving amino acids commonly found at that position are likely tolerated. This method is consistent with research indicating that the effects of substitutions, particularly between hydrophobic and hydrophilic amino acids, can vary significantly, with some changes being well-tolerated while others may be detrimental (Barrett and Pollard, 2012; Weille and Eske, 2013).

CONCLUSION AND RECOMMENDATION

The findings of the study indicate that; The sequence length of base pairs (bp) for the FSHR genes varies between 2471 bp and 3516 bp, both within and among different species. The FSHR genes in chickens, ducks, and geese exhibit polymorphism, featuring a variety of mutations that can be either neutral (beneficial) or harmful (deleterious). The evolutionary analysis of the FSHR genes revealed that ducks and geese are the closest relatives. Based on the study's findings, the following recommendations were made for further discussion; Further genetic studies conducted more extensive genetic analyses across a wider range of avian species to better understand the evolutionary dynamics and functional implications of FSHR gene variants. Investigate the physiological impacts of the identified deleterious FSHR gene variants to determine their effects on reproductive performance and potential breeding strategies. Utilizing genetic information in conservation programs to maintain genetic diversity and enhance the reproductive success of endangered avian species and exploring the potential of using FSHR gene variants as markers in selective breeding programs to improve reproductive efficiency and overall poultry production.

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