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INTERROGATING THE CHICKEN GROWTH HORMONE GENE OF SELECTED NIGERIAN CHICKEN BREEDS USING DNA-SEQUENCING DATA

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ABSTRACT

The growth hormone gene is one of the most significant genes in the body. It regulates hormones related to growth, development, and egg production in avian species among other important traits. The study is centred on understanding the cellular, molecular, and biological mechanisms underlying the activity of the gene by interrogating it using the National Centre for Biotechnology Information (NCBI) database. After extraction of genomic DNA in selected Nigerian indigenous chickens (FUNNAB Alpha, Noiler, Frizzled feathered, Fulani ecotype), and an exotic broiler chicken (Cobb 500), it was amplified at intron 3 of the cGH yielding a product of 715bp. These were sequenced and aligned against the chicken genome with the BLAST programme of the NCBI website to verify their identity. A dendrogram for the five studied chickens and five closely matched species after carrying out Basic Local Alignment Search (BLAST) was generated showing their relationship using the facility for generating phylogenetic relationships on the website. Dendrogram for the five studied chickens based on Nei's unbiased genetic distances was also obtained using MEGAX software. The cGH gene identified was subjected to functional enrichment analysis using the STRING database concentrating on ascertaining molecular, cellular, and

biological processes/pathways associated with the gene. Functional enrichment analysis revealed key associated genes of cGH, as well as the processes and pathways linked to the gene. Markedly, the gene's involvement in the regulation of growth, protein, endocrine, cell proliferation, cell signaling, enzyme activity, response to food, and regulation of appetite were highlighted amongst others. The study has contributed to a profound perception of how the gene impacts on growth and development of avian species. Conclusively, the study revealed that a combination of transcriptomic data and pathway analysis could serve as a powerful tool that will be helpful in the unraveling of the complex molecular activities linked to growth in the chickens.
Keywords: Broilers chicken; Growth hormone; DNA-seq; STRING; Functional enrichment analysis.

INTRODUCTION

The growth hormone is one of the hormones secreted by the somatotropic cells of the anterior lobe of the brain pituitary. It together with its associated genes, are documented to have positive influences on animal growth, metabolism, lactation, and reproduction (Breier, 1999). This gene, with its positional and functional capacity, has been widely used as a genetic marker in many farm animal species (Adigun *et al.,* 2021). The gene is located on the 27th chromosome and contains 5 exons and 4 introns with a total length of 4.35 kbp. These exons and introns have been reported to account for the greatest diversity and are correlated with the performance of animals (An *et al.,* 2010; Wickramaratne *et al.,* 2010). Being a polypeptide hormone, it is produced and discharged by the pituitary gland. It has been particularly reported to affect growth traits (growth rates, body weight, maturation, metabolism rates, egg production, reproduction, appetite control, and aging) in broiler chickens (Harvey, 2013). Growth performance and carcass traits are noteworthy traits of economic importance in broiler chickens' production.

The chicken growth hormone (*cGH*) is a 22 kDa protein, comprised of 191 amino acid residues (Hrabia *et al.,* 2008). The *cGH* consists of 4,101 bp (Kansaku *et al.,* 2008), and affects a variety of physiological functions in the chicken (Apa *et al.,* 1994). It is one of the key genes influencing chicken performance traits (Vasilatos-Younken *et al.,* 2000) including the promotion of muscle and bone growth and development, as well as the regulation of the fat content of meat (Zhang *et al.,* 2007; Mazurowski *et al.,* 2015). Studies revealed its involvement also in sexual differentiation and pubertal maturation processes as well as active participation in gonadal gametogenesis,

steroidogenesis, and ovulation (Hull and Harvey, 2001). A greater level of genetic diversity in the *cGH,* was reported by Nie *et al.* (2002) to exist in the less artificially selected indigenous or native chicken breeds compared to the commercial breeds.

Growth is a trait that is primarily, controlled by polygenic genes. Some of the genes like the Signal Transducer and Activator of Transcription (STAT) gene, which contains 5 conserved domains and occurs in two isoforms (*STAT5A* and *STAT5B*), are associated with growth (Hennighausen *et al.,* 2008; Zhao *et al.,* 2012); yet another like the Bone Morphogenetic Proteins (BMPs), belonging to a bigger subclass of the Transforming Growth Factor-β (TGF- β) performs an important part in ovarian physiology; its isoform (*BMPR-IB*) influences ovulation rate and hence, production of egg (Zhang *et al.,* 2008). Lots of research has been carried out on how genes, *cGH* inclusive, have significantly affected traits of importance (Okumu *et al.,* 2017; Adigun *et al.,* 2021). Okumu *et al.* (2017) for instance, analyzed different indigenous chicken populations based on genetic characterization using microsatellite markers and/or other modern techniques in Kenya. Adigun *et al.* (2021) evaluated the polymorphism and genetic diversity of *cGH* gene in selected chicken breeds in Nigeria and reported the existence of polymorphic variants in them. While these reports are not exhaustive, additions to the body of knowledge are highly welcome because the discovery of genetic diversity will provide opportunities for genetic improvement. However, there's a need for an initial assessment of candidate genes linked to these diversities (Pagala *et al.,* 2017). In Nigeria, such information is insufficient or at worst even nonexistent. Hence, the present study is aimed at interrogating the *cGH* gene of selected Nigerian indigenous chicken breeds using DNA-seq data.

METHODS AND METHODS

Study area

The study was carried out at the poultry farm of the Department of Animal Production, Federal University of Technology, Minna, Niger state, Nigeria located in the Southern Guinea Savanna zone. Its latitude, longitude, annual rainfall and temperature regimes, and altitude are as described by Ojimaduka *et al.* (2020). The study was carried out using five chicken breeds commonly reared in Nigeria (Fulani ecotype, Frizzle feathered, Noiler, FUNNAB Alpha broilers, Cobb 500). The Noiler, FUNNAB Alpha broilers, and Cobb 500 chickens were managed intensively, while the Fulani ecotype and Frizzled feathered chickens were managed extensively.

Blood sample collection

Blood samples (5 ml) were collected from the brachial vein of the chickens using separate 5 ml syringes to prevent cross-contamination. The blood samples were collected into separate Ethylene Di-amine Tetra-acetic Acid (EDTA) bottles and stored in ice packs for onward transfer to the laboratory where genomic DNA was extracted. DNA extraction was done at African Biosciences Ltd., Ibadan, Oyo State, Nigeria, using a gSYNCTM DNA extraction kit (Geneaid) according to the manufacturer's protocol. The extracted DNA quantity and quality were checked by using a spectrophotometer and agarose gel electrophoresis, respectively. The primers used for the study were designed using the NCBI website (Table 1). The Polymerase Chain Reaction (PCR) products obtained after DNA extraction were sequenced and then aligned against the chicken genome with the BLAST programme of the National Centre for Biotechnology Information (NCBI) website (http://ncbi.nlm.nih.gov) to verify their identity. Before sequencing, the extracted DNA was cleaned to ensure that the genes were not contaminated with impurities; this was done using a DNA clean and concentrator kit using the manufacturer's protocol (ZYMO Research).

Sequencing of the genomic DNA

The procedures used in genomic DNA extraction, polymerase chain reaction, and DNA sequencing had been explained previously by Adigun *et al.* (2021). The procedure includes the use of primers (Table 1) for PCR to amplify specific DNA regions from the extracted genomic DNA. The products obtained were subjected to Sanger sequencing and used to determine the nucleotide sequence of the PCR products. The sequencing was followed by sequence alignment whereby the sequenced DNA fragments were aligned against the chicken genome using the BLAST programme on the National Centre for Biotechnology Information (NCBI) website. This alignment was performed to verify the identity of the DNA fragments and to compare them with existing genomic data.

Table 1. Primer used for the Study

5'TCAGTACGCAGACCTACCCTC3'	Forward
5'TGCACATCATGTCCCACGTTT3'	Reverse

Data analysis

The *cGH* gene obtained from the studied chickens, including DNA, was compared with what was obtainable in the NCBI information gene bank by using the BLAST, and similar sequences and nucleotides were investigated. A dendrogram for the five studied chicken breeds and the five closely matched species following blasting on the NCBI website was generated to show the genetic relationship using a facility for generating phylogenetic relationships on the website. A dendrogram for the five studied chicken breeds based on Nei's unbiased genetic distances, using the Unweighted Pair Group Method with Arithmetic mean (UPGMA), was generated to show the populations' genetic distances using MEGAX software. The *cGH* gene identified was subjected to functional enrichment analysis using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database focusing on identifying biological processes and pathways associated with the genes.

RESULTS

Extracted DNA, PCR products

The agarose gel electrophoresis results of the isolated genomic DNA of selected chicken breeds in Nigeria and of the PCR amplified *cGH* are presented in Figures 1 and 2. The amplified products appeared as clear single bands, and occupied the position with approximately 700 bp of amplicon size, with no variation in sizes between the chicken breeds studied.

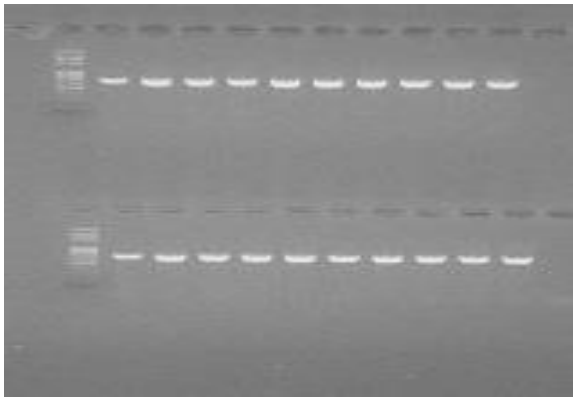

Figure 1. Isolated genomic DNA of the chickens

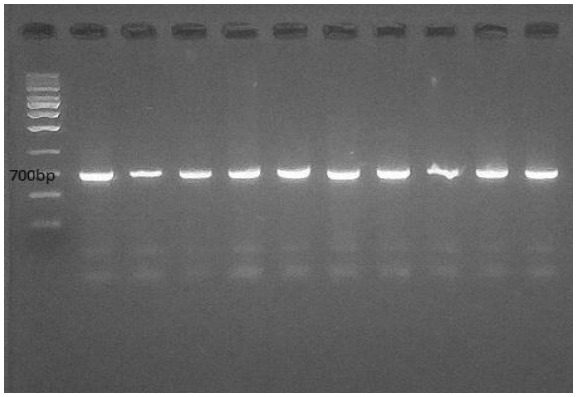

Figure 2. Amplified PCR products of the *cGH*

Scoring of similarity and matching rate of sequences of different chicken genotypes

The scoring of similarity and matching rate of the different sequences of the different chicken genotypes with the reference genotype, and the next five closely related genotypes (*Meleagris gallopova, Rollulus rouloul, Tetrao urogalus, Lagopus leucura, Somateria mollisima*) is given in Table 2. The maximum scores were all >1200 and the *cGH* fragments for all the chickens accessed were >99 % in homology with the reference chicken's genomic sequence (*Gallus gallus* accession no. AY461843.1). The similarity of each of the chicken breeds studied to the five closely related genotypes on the NCBI database was in the following order; *Rollulus rouloul* > *Meleagris gallopova* > *Lagopus leucura* > *Tetrao urogalus* > *Somateria mollissima.*

Phylogenetic relationship between the selected chicken breeds, the reference Gallus gallus gene, and the five most closely related genotypes

Figure 3 shows the phylogenetic relationship among the selected chicken breeds, the reference *Gallus gallus* gene, and the five most closely related genotypes. The phylogenetic relationships among each of the studied breeds, the reference breed, and the five most closely related genotypes were similar. Each of the selected breeds is represented by the unknown in the figure. Six different clades were observed, with the farthest being that between the genotypes and *Somateria mollissima.* Figure 4 shows the phylogenetic relationship between the selected chicken breeds only (Adigun *et al.,* 2021). Two clades were observed in the studied birds.

Functional enrichment of the cGH gene

The functional enrichment of the *cGH* gene showed its association with certain genes and biological processes (Figure 5). The associated genes of the *cGH* are: Growth Hormone Releasing Hormone gene (*GHRH*), Neuropeptide Y Receptor gene (*NPY2R*), Pancreatic Polypeptide gene (*PPY*), Proopiomelanocortin gene (*POMC*), Glycoprotein hormones, alpha polypeptide gene (*CGA*), Insulin-like Growth Factor 1 gene (*IGF1*), Growth Hormone (*GH*), Gherelin and obstatin prepropeptide gene (*GHRL*), Growth Hormone Receptor gene (*GHR*), Prolactin gene (*PRL*) and Prolactin Receptor gene (*PRLR*). The genes are associated with many biological processes at a False Discovery Rate (FDR) of between 0.000075 to 0.024.

DISCUSSION

The amplified gene products obtained in the study were consistent with the expected target fragments and all had a good specificity, which was directly analyzed through the PCR-RFLP technique. The present findings on the *cGH* gene in terms of product size, are in agreement with earlier reports; Muin and Lumatauw (2013) in Indonesia native chickens' population, and Bingxue *et al*. (2003) in F2 chickens (from broilers x Silky crossing in China), respectively. However, others (Khoa *et al.*, 2013; Makhsous *et al*., 2013; Rahmadani *et al.*, 2014; Saikhom *et al.*, 2017) reported product sizes of 563 bp, 1164 bp, 367 bp, and 713 bp, respectively. While some of these reported lower product sizes, others observed higher product sizes. The differences in the base pairs of the PCR fragments in the current study when compared to those reported by the authors cited above, suggest the possibility of insertion/duplication of the sequences.

The *cGH* fragments for all the chickens studied obtained more than 99 % homology with the reference chicken's genomic sequence (accession no. AY461843.1) of *Gallus*. This means that the percentage of genetic bases similar to the reference sequence is very high. The lower values obtained with *Somateria mollissima* however, is a reflection of the genetic distance between the breeds studied and this species. This shows that *Somateria mollissima* became detached from the others quite a long time ago along the evolutionary journey. The higher the similarity percentage, the closer the relationship; meaning the co-ancestry is high either because of a speciation occurrence, a duplication consequence, or a lateral (horizontal) gene transfer (Koonin, 2005). This is evidence that the genotypes are closely related by evolutionary changes emerging from a common ancestor. This is further affirmed by the phylogenetic map, where the closest relationship was between the individual genotypes (unknown; Figure 4) and the reference sequence. The farthest distance between the studied genotypes and *Somateria mollissima* therefore, is a clear indication of how distant their homologs are. This is a reflection of the number of genetic bases that have become different over time in the evolutionary journey.

The phylogenetic relationship between the studied genotypes divided them into two groups. While the Fulani ecotype chicken was found in the same group with the FUNNAB Alpha and Cobb 500 (both chickens with a high percentage of exotic blood), the Noiler and Frizzled feathered were in the same group. Tiamiyu (1999) opined that the Fulani chicken was developed from exotic cockerels (Rhodes Island Red) bloodlines used in previous improvement programmes when they

were used to mate with indigenous hens. The Noiler and Frizzled feathered chickens are both dual-purpose birds and this might have accounted for their closeness and possible co-ancestry.

Functional enrichment analysis is a powerful tool used in the discovery of the molecular, biological, and cellular processes or functions associated with a particular gene. This is because it helps in bringing out the protein–protein interaction networks that are important ingredients for the understanding of cellular processes in organisms. Such interaction networks are useful in sifting through and evaluating functional genomics data; this helps in providing an intuitive platform for explaining and interpreting of structural, functional, and evolutionary properties of proteins. This kind of exploration is capable of suggesting novel guidelines for future experimental research and providing cross-species predictions for efficient gene interaction mapping (Schwartz *et al.,* 2008). The gene under study (*cGH*) has been linked to function in the extracellular space and molecularly, it aids neuropeptide receptor binding hormone activity, protein-coupled receptor binding, and in signaling receptor binding. Its biological functions are multifaceted including regulation of glucocorticoid secretion, positive regulation of growth hormone secretion, response to food/regulation of appetite, insulin-like growth factor receptor signaling pathway,

Table 2: Scoring of similarity and matching rate of sequences of different chicken genotypes in the case of *cGH* gene

Genotype	FUNNAB Alpha		Cobb 500		Noiler		Fulani ecotype chicken		Frizzled Feathered chicken	
Related genotypes	**Max Score**	**Identity (%)**	**Max Score**	**Identity (%)**	**Max Score**	**Identity (%)**	**Max Score**	**Identity (%)**	***Max Score**	**Identity (%)**
Gallus gallus AY461843.1	1277	99.58	1272	99.44	1257	99.02	1268	99.30	1272	99.58
Meleagris gallopova (Turkey) OW982277.1	1006	90.50	1011	90.63	1000	90.37	1006	90.50	997	90.22
Rollulus rouloul (Creasted Partridge) EF521549.1	928	91.64	932	91.80	921	91.50	932	91.80	919	91.33
Tetrao urogalus (Western Capercaillie) (OX596320.1	850	85.95	864	86.37	857	86.25	864	86.37	859	86.23
Lagopus leucura (White-tailed Ptarmigan) XR_006181562.1	680	86.40	680	86.40	689	86.75	689	86.75	680	86.40
Somateria mollissima (Common Eider) OX598326.1	545	75.39	549	75.65	541	75.79	549	75.52	545	75.39

*Max = maximum

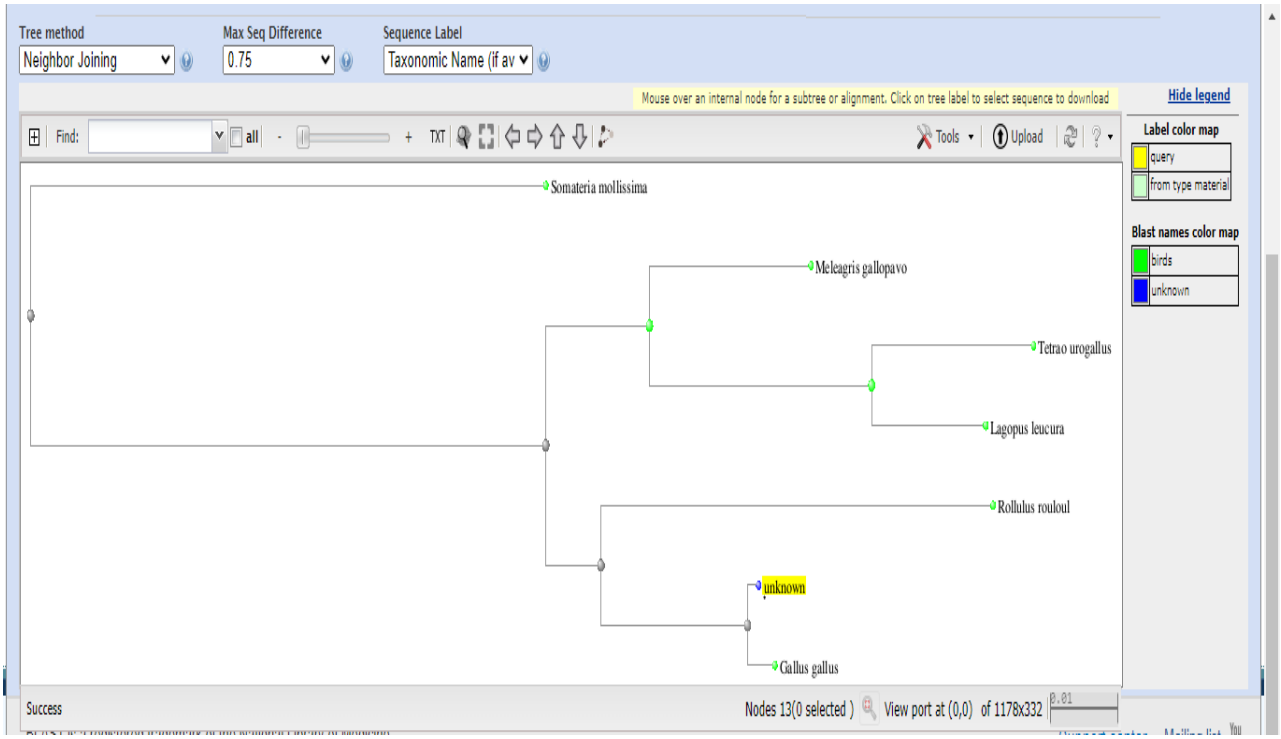

Figure 3: Phylogenetic relationship between the selected chicken breeds (unknown) and the closest neighbours

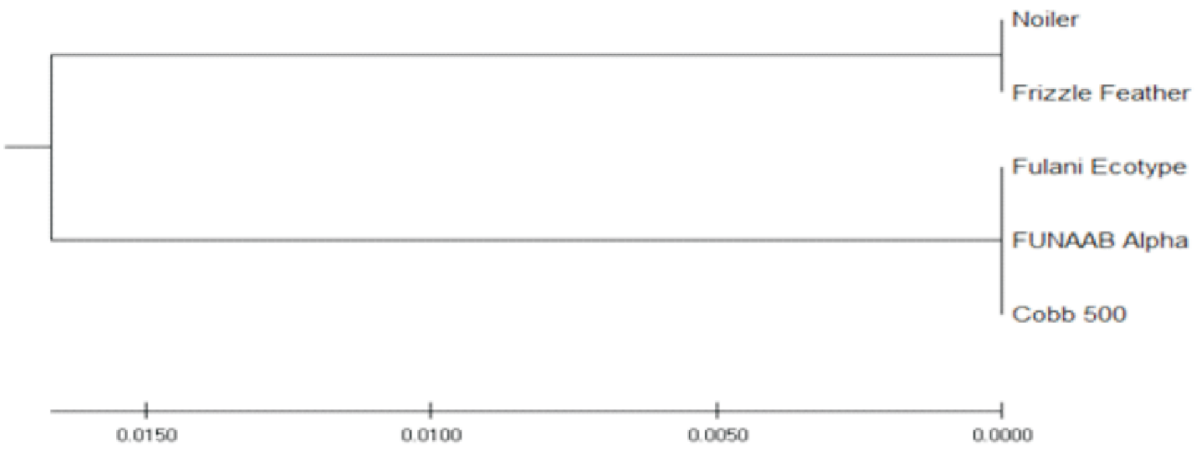

Figure 4: Phylogenetic relationship between the chicken breeds at the *cGH* gene locus

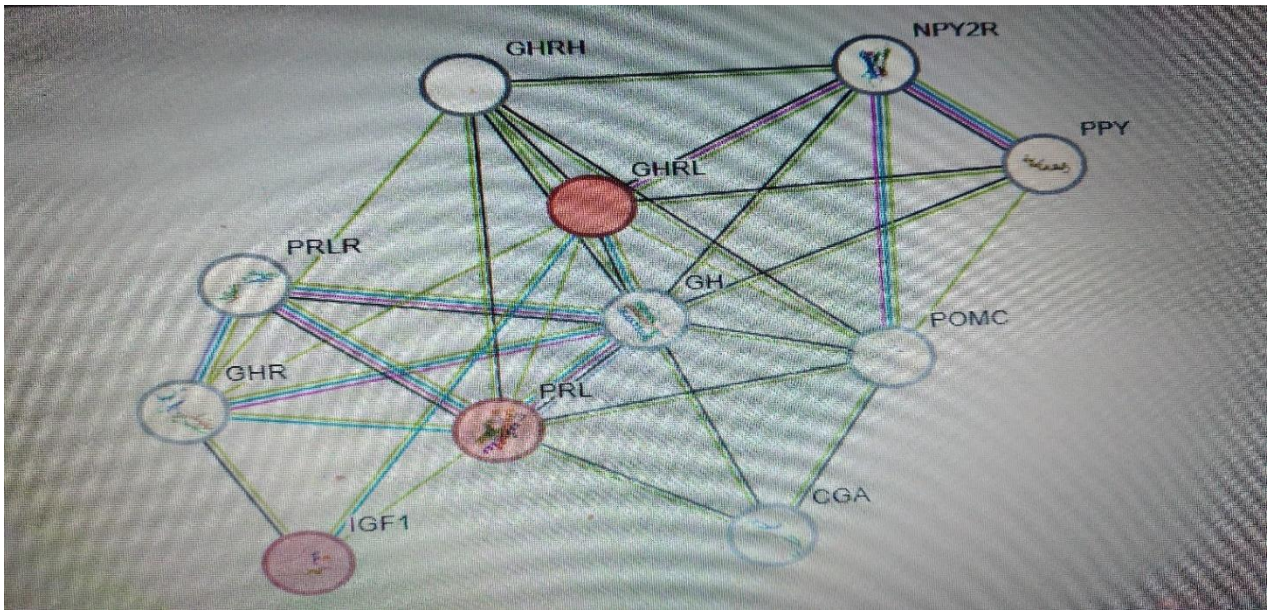

Figure 5: Genes related to the *cGH* following functional enrichment analysis

Key: *GH* = growth hormone gene, *GHRH* = growth hormone releasing hormone gene, *NPY2R* = neuropeptide Y receptor gene, *PPY* = pancreatic polypeptide gene, *POMC* = proopiomelanocortin gene, *CGA* = glycoprotein hormones, alpha polypeptide gene, *IGF1* = insulin-like growth factor1 gene, *GHRL*= ghrelin and obstatin prepropeptide gene, *PRLR* = prolactin receptor gene, *GHR* = growth hormone receptor gene, *PRL* = prolactin gene.

positive regulation of receptor signaling pathway via JAK-STAT, growth hormone receptor signaling pathway, regulation of endocrine process, negative regulation of insulin secretion, regulation of multicellular organism growth, negative regulation of hormone secretion, tumor necrosis factor production, and endothelial cell proliferation, positive regulation of peptide hormone secretion, and control of the neuropeptide signaling pathway. Other biological functions are positive regulation of peptidyl-tyrosine phosphorylation, response to nutrient levels, response to hormones, enzyme-linked receptor protein signaling pathway, positive regulation of cell population proliferation, metabolic processes, cell communication, signal transduction, and G protein-coupled receptor signaling pathway. With all these important biological, cellular, and molecular functions, it means any mutation in the gene (positive or negative) will likely have an impact on the functions that the gene and its associated genes control. This has been alluded to by several authors (Kastrup *et al.*, 1978; Schmidt *et al.*, 1996; Ariyasu *et al.*, 2005; Liu *et al.*, 2016;

Aguiar-Oliveira *et al.*, 2017, 2018; Aguiar-Oliveira and Bartke, 2019). The application of functional analysis in the understanding of modern biology and medicine (Barabasi and Oltvai, 2004; Hu *et al.*, 2016; Conte *et al.*, 2020) can therefore, not be discountenanced. Their usage will enable not only the interpretation of molecular functions via the 'guilt-by-association' principle as postulated by Tian *et al.* (2008) and Cowen *et al.* (2017), but also, allow for the classification of modularity in biological processes (Choobdar *et al.*, 2019; Serban, 2020) and may serve as a benchmark for deeper learning (Camacho *et al.*, 2018; Gligorijevic *et al.*, 2018). Other applications are in the area of drug target detection or drug repurposing (Lotfi Shahreza *et al.*, 2018; Pushpakom *et al.*, 2019). It will also come in handy in interpreting genomic variation in various species (Wu *et al.*, 2018).

CONCLUSION

Investigation of the *cGH* gene has shed some light on its complex cellular, biological, and molecular functions. Identification of some of the genes connected with growth and development in the chicken could accelerate exploring into the dynamics and functional pathways associated with the gene. Integrating transcriptomic data and pathway analysis as was done in the study will help in deciphering the complex activities of *cGH* and its associated genes.

Competing Interests

The authors declare that they have no competing interests associated with this article.

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