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Pituitary transcriptome profile from laying period to incubation period of Changshun green-shell laying hens

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Abstract

Background Incubation behaviour, an instinct for natural breeding in poultry, is strictly controlled by the central nervous system and multiple neuroendocrine hormones and neurotransmitters, and is closely associated with the cessation of egg laying. Therefore, it is essential for the commercial poultry industry to clarify the molecular regulation mechanism of incubation behaviour. Here, we used high-throughput sequencing technology to examine the pituitary transcriptome of Changshun green-shell laying hen, a local breed from Guizhou province, China, with strong broodiness, in two reproductive stages, including egg-laying phase (LP) and incubation phase (BP). We also analyze the differences in gene expression during the transition from egg-laying to incubation, and identify critical pathways and candidate genes involved in controlling the incubation behaviour in the pituitary.

Results In this study, we demonstrated that a total of 2089 differently expressed genes (DEGs) were identified in the pituitary, including 842 up-regulated and 1247 down-regulated genes. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that steroid biosynthesis pathway and neuroactive ligand-receptor interaction were significantly enriched based on DEGs commonly identified in pituitary. Further analysis revealed that SRC, ITGB4, ITGB3, PIK3R3 and DRD2 may play crucial roles in the regulation of incubation behaviour.

Conclusions We identified 2089 DEGs and the key signaling pathways which may be closely correlated with incubation in Changshun green-shell laying hens, and clarified the molecular regulation mechanism of incubation behaviour. Our results indicate the complexity and variety of differences in reproductive behaviour of different chicken breeds.

Keywords Changshun green-shell laying hens, Pituitary, Transcriptome analysis, Laying period, Incubation period

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Introduction

Incubation behaviour, also colloquially known as broodiness, is a behaviour commonly occurring in domestic poultry. In domestic chickens, incubation behaviour consists of persistent nesting, turning and retrieval of eggs, characteristic clucking, and defense of the nest [1, 2]. It can cause atrophy of the fallopian tubes and ovaries, and the subsequent cessation of egg laying, leading to economic losses for the domestic poultry industry [1]. Although the mechanism of incubation behaviour has been extensively investigated in domestic chickens, the exact regulatory mechanisms remain elusive. Usually, incubation behaviour is known to be strictly controlled by the central nervous system and is closely associated with multiple neuroendocrine hormones and neurotransmitters.

During the transition from egg-laying to incubation in domestic chickens, the levels of hormones change markedly, and prolactin (PRL) is considered to be a main factor induced during incubation. The onset and maintenance of incubation behaviour in chickens are demonstrated to be closely correlated with a characteristic increase in plasma PRL level [3–7]. Meanwhile, the concentrations of luteinizing hormone (LH) and steroid hormones in plasma decrease dramatically during incubation, leading to regression of the ovary [5]. Once the young chicks have hatched, the plasma PRL level subsequently declines dramatically, and the concentrations of LH and estradiol increase gradually [5, 8–10]. Increased PRL and decreased LH secretion in incubating chickens are found to be correlated with increased hypothalamic neuropeptide vasoactive intestinal polypeptide (VIP) and decreased hypothalamic gonadotropin-releasing hormone (GnRH), respectively. The hypothalamic VIP is a physiological PRL-releasing hormone in chickens. It can not only increase the gene expression of *PRL* in pituitary gland, but also induce the release of PRL by acting directly on the the lactotrophs of pituitary gland [11–14]. In addition, the neurotransmitters are also thought to be involved in the regulation of avian incubation behaviour. The neurotransmitter dopamine (DA) has both an inhibitory and stimulatory effect on the secretion of avian PRL, depending on the subtype of DA receptor [15, 16]. However, 5-hydroxytryptophan (5-HTP) and dynorphin are reported to stimulate VIP and PRL release, respectively [17, 18].

In addition to neuroendocrine control, many investigations on the genetic control of incubation behaviour have been reported in the past decades. However, these investigations appear to have led to conflicting observations. Previous studies have extensively reported that incubation behaviour is a polygenic trait, and is controlled by more than one independent autosomal gene [19, 20]. While, subsequent investigations indicated that incubation behaviour might be controlled, at least to a large extent, by Z-linked genes, which seems to be contradictory to previous findings [21, 22]. Interestingly, the quantitative trait loci (QTL) analysis indicated that genetic loci affecting incubation behaviour are not located on the Z chromosome, but on chromosomes 1, 5, 8, 13, 18 and 19 and linkage group E22C19W28 [23]. Overall, it is now generally accepted that more than one locus controls the incubation behaviour in domestic chickens.

Changshun green-shell laying hen is an indigenous breed of chicken in Guizhou province, China. It is known to be a dual purpose egg- and meat-type chicken, and their eggs are considered to be superior in local markets for their better appearance, higher contents of protein, better amino acid composition, and lower fat and cholesterol content [24]. Although its strong tendency for broodiness significantly reduces egg production, and has restricted development of the Changshun chicken industry, the stable laying-incubation cycles and easily identifiable characteristics of broodiness make it an ideal model to study incubation behaviour and the molecular regulatory mechanism of poultry broodiness.

In recent years, RNA sequencing (RNA-Seq) has been widely used to investigate the dynamic gene expression profiles of key genes in the hypothalamic-pituitary-gonadal (HPG) axis of poultry between the laying period and incubation period [25–27]. A total of 110 differentially expressed genes (DEGs) were screened in the pituitary between laying and nesting geese, and the PRL, oxytocin-neurophysin (OXT), chordin-like protein 1 (CHRDL1) and growth hormone (GH), expressed in the pituitary gland, are considered as new candidate molecules involved in incubation in geese [25]. In ducks, 398 DEGs were identified in pituitary during the transition from egg-laying to incubation, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis further indicated that neuroactive ligand-receptor interaction pathway, calcium signaling pathway, and response to steroid hormones biological process are critical for the transition from egg-laying to incubation phase of ducks [27]. Considering the complexity and variety of differences in reproductive behaviour of different poultry breeds, it is essential to understand the genetic basis of the transition from egg-laying to incubation phase of chickens at transcriptome level.

A large number of studies have focused on the identification of potential genes and gene regulatory networks associated with high rates of egg production in chicken pituitary [28, 29]. However, there are few studies on pituitary transcriptome analysis of incubation behaviour in chickens. Here, we systematically examined the pituitary transcriptome of Changshun green-shell laying hens at LP and BP through high-throughput sequencing and

concentrated on the analysis of the differences in gene expression during the transition from LP to BP. Candidate genes involved in incubation were screened out, and related pathways were evaluated through KEGG enrichment and GO enrichment analysis. This study further reveals the complexity and variety of differences in reproductive behaviour of different chicken breeds and provide new insights into poultry reproductive behaviour.

Materials and methods

Ethics statement

The animal experiments were performed under the guidelines published in the Guide for the Care and Use of Laboratory Animals of the Ministry of Science and Technology of the People's Republic of China. All methods strictly follow ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines 2.0. The experimental procedures in this study were approved by the Animal Ethics Committee of the College of Biological Science and Agriculture, Qiannan Normal University for Nationalities, Duyun, China (AEC No. QNUN2020018).

Animals and sample preparation

The experimental Changshun green-shell laying hens were obtained from Changshun Sanyuan Agricultural Development Co., Ltd., China and were then raised in the poultry breeding farm of Qiannan Normal University for Nationalities according to the standard procedure. 100 females and 15 males were reared on the floor with litter and artificial nest and exposed to natural temperature conditions. An infrared video camera was set up above laying nest. The incubation behaviours were then recorded daily in the annual reproductive cycle. The individuals with high fertility were deemed to be LP birds. The hens which sat in the nest while exhibiting characteristic clucking, aggressive or defensive behaviour for at least one week were allocated as the BP group. Six hens from LP and BP stages were randomly chosen according to their behaviours and the ovary and anterior and posterior pituitary gland samples were quickly collected after euthanization by exsanguination under sodium pentobarbital anesthesia (60 mg/kg), respectively. The morphologic characteristics of the ovaries in the study were mainly used to further evaluate individual physiological stage of chickens (Fig. 1). All the anterior and posterior pituitary samples were immediately frozen in liquid nitrogen and then stored at -80 °C until total RNA extraction for transcriptome sequencing. Three pituitary samples from each of the LP and BP groups were randomly chosen for transcriptome sequencing according to the morphologic characteristics of the ovary.

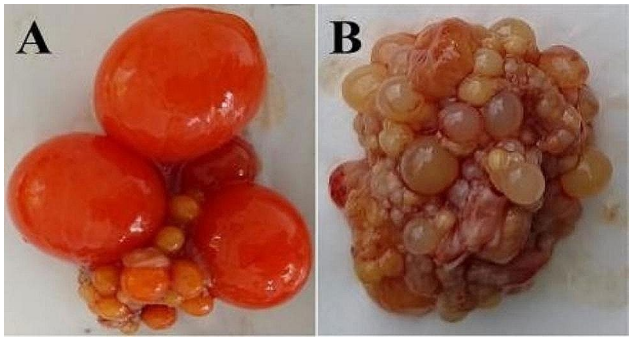

Fig. 1 Ovary morphology of hens at LP (A) and BP (B)

RNA extraction, cDNA library construction, and mRNA sequencing

Total RNA from three individuals in each of the LP and BP groups was extracted from the pituitary samples using the Trizol reagent (Life technologies, California, USA), according to the manufacturer's instruction manual. The concentration and quality of the total RNA were determined using NanoDrop 2000 (Thermo Fisher Scientific, Wilmington, DE). RNA integrity was evaluated using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). Sequencing libraries were generated using Hieff NGS Ultima Dual-mode mRNA Library Prep Kit for Illumina (Yeasen Biotechnology (Shanghai) Co., Ltd.) following manufacturer's recommendations. The libraries were sequenced on an Illumina NovaSeq6000 platform using 150 bp paired-end reads, according to the manufacturer's instructions.

Data analysis

The raw reads were further processed with a bioinformatics pipelinetool, BMKCloud (www.biocloud.net) online platform. Raw sequences were firstly transformed into clean reads through removing adaptor sequences and discarding low-quality sequences. Hisat2 tools software (v2.2.1, -dta -p 6 -max-intronlen 5,000,000) was then used to map against the chicken reference genome (Gallus_gallus.GRCg6a_release106.genome.fa) [30]. Both known and novel transcripts from Hisat2 alignment results were detected by the method of the StringTie Reference Annotation Based Transcript (v2.2.1, -merge -F 0.1 -T 0.1) [31]. The KEGG (http://www.genome.jp/kegg/), National Center for Biotechnology Information for non-redundant proteins (ftp://ftp.ncbi.nih.gov/blast/db/), Eukaryotic Orthologous Groups of proteins (http://www.ncbi.nlm.nih.gov/KOG/), Protein family (http://pfam.xfam.org/), GO (http://www.geneontology.org/) and Swiss-Prot (http://www.uniprot.org/) databases were used to annotate the gene function. The principal component analysis (PCA) was applied to assess the similarity between the samples.

Identification of DEGs

Differential expression analysis of between the two groups was performed using DESeq2 (v1.30.1) [32]. DESeq2 provides statistical routines for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting *P* values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted *P*-value < 0.05 & Fold Change ≥ 1.5 found by DESeq2 were assigned as differentially expressed.

KEGG pathway, GO enrichment analysis and protein-protein interaction

GO enrichment analysis of DEGs was implemented by the clusterProfiler package (v3.16.1) based Wallenius non-central hyper-geometric distribution, and KOBAS software (v3.0) was used to test the statistical enrichment of DEGs in KEGG pathways [33, 34]. The protein-protein interaction (PPI) analysis was performed using Cytoscape [35].

Gene expression analysis by quantitative real-time PCR

Using total RNA, 1 μg was reverse transcribed to cDNA using the Prime Script RT reagent Kit (Takara, Dalian, China). Four genes of interest, including *PRL*, *BMP5*, *DHCR7* and *SC5D*, were selected to validate the RNA-seq results. *Beta-actin* was chosen as an internal reference gene for the normalization of expression levels. The primers used in the quantitative real-time PCR (qRT-PCR) were designed using Primer 5 (Table S1). Each assay was carried out in triplicate. The relative mRNA expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method [36].

Statistical analysis

Statistical analysis was performed using the R software (v4.0.2, R Development Core Team 2019). Data were analyzed using the Student's t-test after testing for the homogeneity of variance with Levene's test. All data are presented as the mean ± SD, and statistical significance is shown as $^{*}P < 0.05$.

Results

RNA sequencing quality assessment

A total of six libraries were constructed from the pituitary and sequenced using Illumina NovaSeq platform. The reads of transcriptome data and their statistical analysis are shown in Table 1. The clean reads from each library averaged more than 19 million. The base percentage of the Q20 (base sequencing error probability < 1%) and Q30 (base sequencing error probability < 0.1%) ranged from 98.15 to 98.46% and 94.72–95.42%, respectively, indicating high accuracy of the sequencing. The GC content of all pituitary samples ranged from 47.98 to 49.54%. The above results showed that the sequencing data was suitable for subsequent data analysis.

Transcriptome alignment

As shown in Table 1, the mapping rate of the clean reads from six pituitary samples ranged from 93.30 to 94.88%. The percentage of multiple and uniquely mapped reads in clean reads ranged from 1.84 to 3.34% and 90.80–92.22%, respectively. The results indicated that the transcriptome data were reliable and suitable for subsequent analysis.

Differentially expressed genes

Principal component analysis (PCA) was first conducted to analyze the six samples in LP and BP. As shown in Fig. 2A, the pituitary samples in LP and BP groups were separated from each other, indicating an obvious difference in mRNA expression between these two groups. The correlation coefficient of gene expression levels between samples demonstrated good sample repeatability in each group (Fig. 2B). A comparison of the gene expression showed that a total of 2089 DEGs were identified in the pituitary, including 842 up-regulated and 1247 down-regulated genes in the BP group (Fig. 3 and Table S2). Subsequently, hierarchical clustering analysis was carried out on the DEGs. We observed that the pituitary samples of LP and BP groups were distributed in different sub-clusters, but the three biological replicates from each group all clustered closely together. The heatmap visually reflected the expression patterns of the genes in the samples, indicating the reliability of screened DEGs (Figure S1).

Table 1 Quality metrics of transcripts in pituitary

Sample	Clean reads	Clean bases	Q20 (%)	Q30 (%)	GC content (%)	Total mapped	Multiple mapped	Uniquely mapped
LP1	21,699,331	6,489,415,358	98.20	94.77	49.15	20,291,871 (93.51%)	398,685 (1.84%)	19,893,187 (91.68%)
LP2	23,189,413	6,932,116,122	98.33	95.10	49.24	21,704,639 (93.60%)	461,979 (1.99%)	21,242,661 (91.60%)
LP3	19,290,472	5,767,577,132	98.15	94.72	49.54	17,998,306 (93.30%)	440,208 (2.28%)	17,558,098 (91.02%)
BP1	24,468,336	7,318,629,774	98.43	95.27	47.98	23,215,360 (94.88%)	741,255 (3.03%)	22,474,105 (91.85%)
BP2	20,122,438	6,020,221,454	98.46	95.42	48.47	18,942,509 (94.14%)	671,406 (3.34%)	18,271,104 (90.80%)
BP3	21,727,047	6,500,165,130	98.40	95.27	48.79	20,484,626 (94.28%)	650,102 (2.99%)	19,834,525 (91.29%)

Note: Q20 and Q30 represent error rate less than 1% and 0.1%, respectively. GC content presents the percentage of G and C bases in clean data

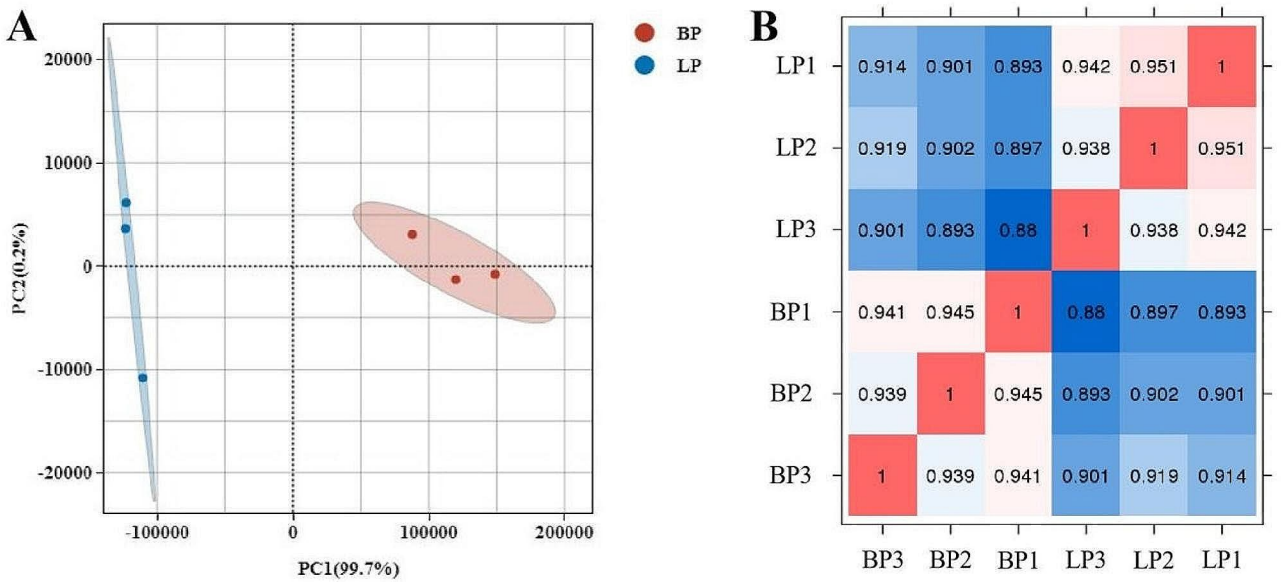

Fig. 2 Features of sequencing data. (**A**) PCA score plot of pituitary transcriptomes. Blue and red nodes represent individuals from egg-laying phase (LP) and incubation phase (BP), respectively. (**B**) Pearson correlation analysis of LP and BP samples. LP1, LP2 and LP3 are pituitary samples of egg-laying phase hens, and BP1, BP2 and BP3 are pituitary samples of incubation metaphase hens

KEGG pathway and GO enrichment analysis

The KEGG pathway enrichment analysis was performed to further identify the major biochemical, metabolic, and signal transduction pathways of the DEGs. The top 20 significantly enriched KEGG pathways are listed in Fig. 4. The ten most enriched pathways were steroid biosynthesis, focal adhesion, MAPK signaling pathway, neuroactive ligand-receptor interaction, vascular smooth muscle contraction, regulation of actin cytoskeleton, TGF-beta signaling pathway, glutathione metabolism, ECM-receptor interaction and axon guidance. Notably, valine, leucine and isoleucine biosynthesis had the largest enrichment factor. Subsequently, the DEGs were annotated with Gene Ontology (GO) database into three main categories namely biological process (BP), cellular component (CC) and molecular function (MF) and 31 subcategories (Fig. 5 and Table S3). Based on the GO classification, 22 subcategories were assigned to the BP, 10 and 4 subcategories to the MF and CC, respectively (Table S3).

Interaction network construction of DEGs

To further explore the biological characteristics of these DEGs from the pituitary, an analysis of protein-protein interaction (PPI) networks was performed (Fig. 6). The PPI networks were visualized by Cytoscape based on information gained up to 3 levels of functional analysis: fold change of genes, PPI and KEGG pathway. The PPI network of DEGs consisted of 209 nodes and 1252 edges. Functional analysis further indicated that the PPI network was mainly enriched into 10 important pathways including steroid biosynthesis, neuroactive ligand-receptor interaction, MAPK signaling pathway, ECM-receptor interaction, glutathione metabolism, focal adhesion, regulation of actin cytoskeleton, vascular smooth muscle contraction, axon guidance and TGF-beta signaling pathway. The top five genes with the highest degree in the PPI network were *SRC*, *ITGB4*, *ITGB3*, *PIK3R3* and *DRD2*, which may play important roles in mediating the transition from egg-laying to incubation in chickens.

Verification of DEGs by qRT-PCR

To validate the RNA-seq results, four DEGs identified in pituitary, including *PRL*, *BMP5*, *DHCR7* and *SC5D*, were selected for qRT-PCR analysis. While there are clear differences in the expression levels of these four genes, the expression trends determined by the qRT-PCR were consistent with the RNA-Seq results, indicating that the RNA-seq results were reliable (Fig. 7).

Discussion

Broodiness is known to be a complicated maternal behaviour which consists of cessation of egg laying, the incubation of eggs, and care of the young. It therefore becomes advantageous to clarify the molecular mechanism of the broodiness in poultry. The pituitary, as a key component of the HPG system, plays a critical role in chicken reproductive performance. In this study, an analysis of whole pituitary transcriptome differences was performed to explore the molecular mechanism underlying the transition from laying to incubation in chickens. The obtained pituitary RNA sequencing data will help us to further

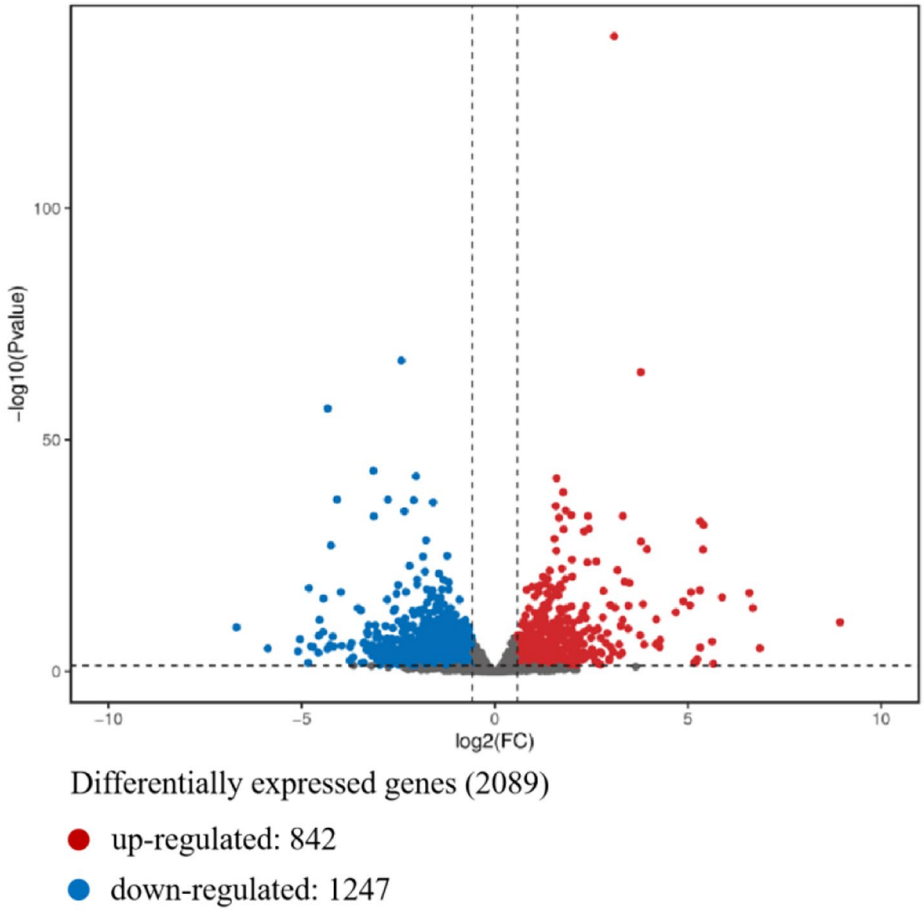

Fig. 3 Volcano map of all expressed genes. The longitudinal and horizontal coordinates represent the statistical significance of the changes in gene expression and the fold changes of genes between the LP and BP groups, respectively. Each dot in the map represents one gene. Red and blue represent significantly up- and down-regulated genes, respectively. Gray represents genes showing no significant difference

understand the biological processes that regulate these two reproductive behaviours.

The incubation behaviour of poultry is mainly affected by the levels of PRL and steroid hormones in plasma. In 1930s, PRL as a hormone of the anterior pituitary was firstly identified to stimulate incubation behaviour in pigeons and chickens [37, 38]. PRL has been known to play a fundamental role in the initiation and maintenance of incubation behaviour in poultry. As expected, transcriptome sequencing revealed that the *PRL* gene was one of the most active DEG in the pituitary, exhibiting an extremely high mRNA level in BP birds. Although it is well established that a characteristic increase of PRL in plasma is closely correlated with the initiation and maintenance of incubation behaviour in domestic and wild birds, the plasma PRL levels seem to be different between precocial and altricial birds. It was reported that the PRL levels in precocial chickens immediately decreased following the hatching of chicks and can be modified by removal of newly hatched chicks from hens [39]. Interestingly, plasma PRL levels in altricial birds seemed to decline more slowly after hatching, presumably because brooding by the parent is imperative for young chicks to survive to fledging.

In addition to PRL, we observed that the expression levels of other hormone-related genes in pituitary such as *PGR*, *RLN3*, *NR5A1* and *GRP* sharply declined in incubating chickens. Similarly, these genes are involved in the initiation and maintenance of incubation behaviour in chickens. PGR as a member of the nuclear receptor superfamily is a specific steroid hormone receptor. The ligand progesterone, a key steroid hormone produced by granulosa cells, can activate PGR and then together they regulate reproductive cycles [40]. RLN3 is the most recently identified member of the relaxin peptide family which may be involved in female reproduction in chickens [41]. It is highly expressed in the pituitary with lower abundance in the hypothalamus, hindbrain, spinal cord and ovary [41]. The expression of *RLN3* is tightly regulated by GnRH, corticotropin-releasing hormone and sex steroid hormone (E2) [41]. The relevant literature suggests that RLN3 might be involved in modulating a range

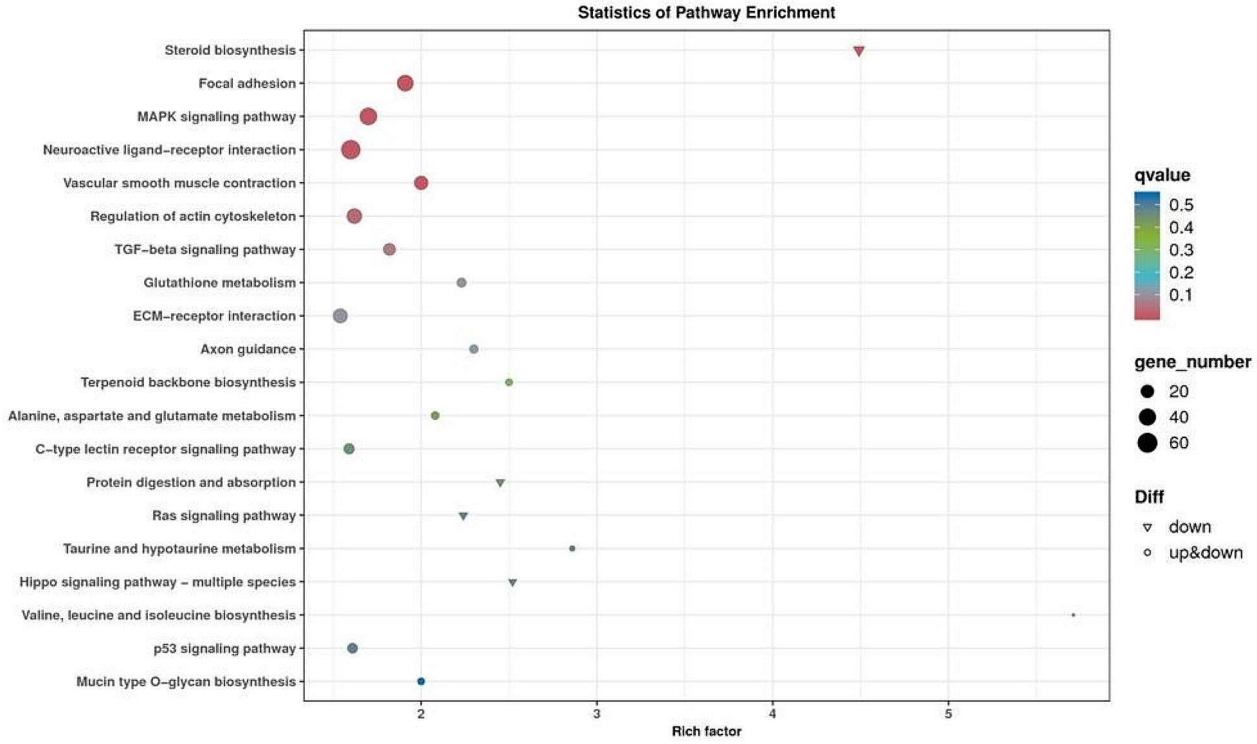

Fig. 4 KEGG pathway enrichment analysis of DEGs in pituitary. Bubble charts represent the top 20 significantly enriched KEGG pathways (with smallest q value). Each circle represents a KEGG pathway. Size of each circle represents the number of DEGs in each pathway and the colour represents the q value of each pathway. The enrichment factor is the ratio of the number of DEGs enriched in the pathway compared to the total number of all annotated genes enriched in this pathway

of interrelated functions such as responses to stress, memory, circadian rhythm, sleep/wake states, food intake, metabolism, satiety and neuroendocrine function [42–47]. Meanwhile, preliminary studies have indicated a modulatory role for RLN3 as a pituitary hormone in reproduction and associated behaviour. Transcriptome analysis showed that RLN3 might be related to the transition from laying to brooding in chickens [27, 48]. Both intracerebroventricular and intraparaventricular administration of RLN3 in animals significantly increased the plasma levels of LH, and this effect was blocked by administration of a peripheral GnRH antagonist [49]. GRP is predominantly and abundantly expressed in the anterior pituitary gland and weakly expressed in other tissues in chickens, including the testes, ovary, proventriculus, telencephalon and hypothalamus [50]. In accordance with their wide distribution, GRP is reported to be involved in many physiological processes in poultry, such as circadian clock, food intake, itch sensation, proventriculus acid secretion, gallbladder motility, crop-emptying rate, pancreatic secretion [50–57]. Additionally, evidence has been accumulating to support the notion that pituitary GRP as a novel hormone in chickens may work together with GRP from other tissues to act in a coordinated fashion to regulate reproduction [50]. NR5A1 is an enigmatic orphan receptor essential for the sexual development and reproduction in mammals [58, 59]. Furthermore, it is known to be involved in the expression of pituitary gonadotropic hormone LH and follicle-stimulating hormone (FSH) and the synthesis of progesterone, androgens, estrogens and steroidogenesis [60].

Steroid biosynthesis plays an important role in the transition from the laying to incubation phases in chickens. It usually decreases sharply during the transition from laying to incubation in poultry. Consistently, the steroid biosynthesis pathway was observed to show the most significant changes during the transition from laying to incubation. In this study, eleven DEGs involved in steroid biosynthesis, including *DHCR7*, *LIPA*, *DHCR24*, *SQLE*, *FDFT1*, *HSD17B7*, *LSS*, *CYP51A1*, *SC5D*, *NSDHL* and *MSMO1*, are observed to decrease sharply during the transition from laying to incubation. Furthermore, the neuroactive ligand-receptor interaction pathway was reported to be important for hormonal regulation in reproductive cycle transitions of duck. Similarly, we found that the expression of DEGs associated with the neuroactive ligand-receptor interaction pathway were observed to be significantly different between LP and BP groups, indicating that neuroactive ligand-receptor interaction pathway might play an important

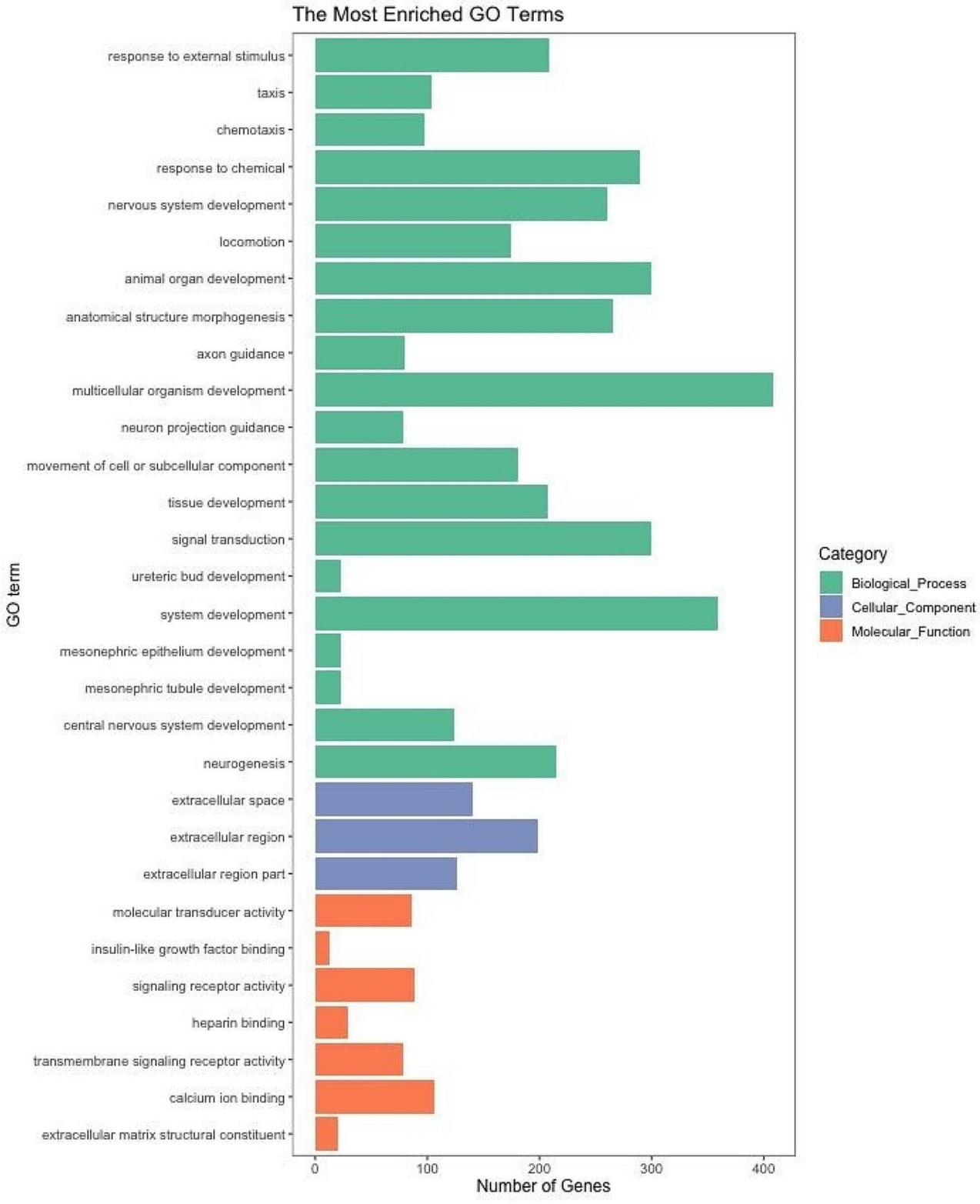

Fig. 5 GO classification of DEGs identified in pituitary. The longitudinal coordinates represent the number of DEGs annotated to the term. The horizontal coordinates represent the GO terms and classifications

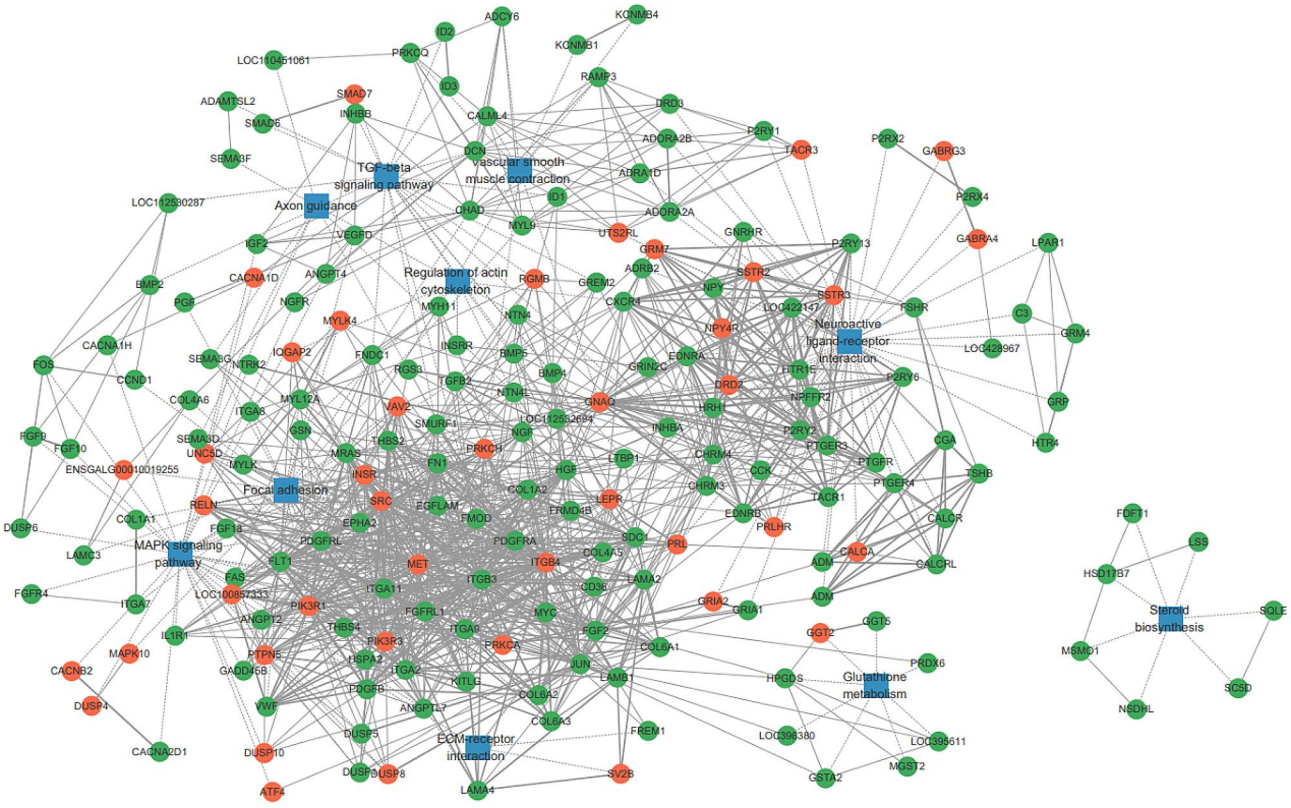

Fig. 6 PPI networks of DEGs in pituitary. Circle nodes for genes/proteins, rectangle nodes for KEGG pathway. In case of fold change analysis, genes/proteins were coloured in red (upregulation) and green (downregulation). Interactions are shown as solid lines between genes/proteins, and edges of KEGG pathway in dashed lines

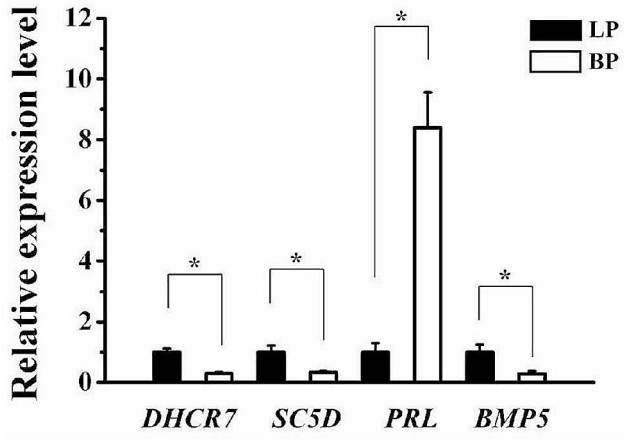

Fig. 7 qRT-PCR validation of DEGs. The relative mRNA expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method. The results were expressed as mean ± SD. * for $p < 0.05$

role in reproductive cycle transitions of poultry. In this study, the DEGs involved in the TGF-β signaling pathway, including *BMP5*, *BMP2*, *BMP4*, *INHBA*, *INHBB*, *GREM2*, *DCN*, *LTBP1*, *FMOD*, *ADAMTSL2*, *SMURF1* and *SMAD6*, were observed to decrease during the transition from laying to incubation. BMP5, a key member of the TGF-β signaling pathway, is reported to participate in fecundity of animals [61]. Meanwhile, BMP5 could affect ovary development by TGF-β signaling pathway activation [62]. BMP2 has been identified as a candidate gene within the hypothalamic-pituitary-gonadal axis responsible for different egg production performance of ducks [63]. It might be involved in the egg-laying performance of poultry via regulating follicle development and ovulation [63]. In addition, the MAPK pathways in the HPG axis are known to be an intracellular serine/threonine kinase signal transduction pathway that is widely distributed in various tissues including hypothalamus, pituitary glands, and ovaries and can be activated by a variety of stimuli. In chicks, there seem to be gender differences in MAPK pathways in HPG axis [64]. It was reported that heat stress can trigger MAPK crosstalk to turn on the hyperosmotic response pathway [65]. During the early brooding stage, high temperature training was thought to aid chicks in surviving warm environments [64].

In conclusion, the pituitary transcriptome in laying and incubating Changshun hens was evaluated in the present study. We identified a total of 2089 DEGs and found that steroid biosynthesis pathway and neuroactive ligand-receptor interaction were significantly enriched. Five candidate genes, including *SRC*, *ITGB4*, *ITGB3*, *PIK3R3*

and *DRD2*, may play crucial roles in the regulation of incubation behaviour in chickens.

Abbreviations

HPG	Hypothalamic-pituitary-gonadal
LP	Egg-laying phase
BP	Incubation phase
DEG	Differently expressed gene
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
PPI	Protein-protein interaction
GnRH	Gonadotropin-releasing hormone
VIP	Vasoactive intestinal polypeptide
LH	Luteinizing hormone
DA	Dopamine
5-HTP	5-hydroxytryptophan
QTL	Quantitative trait loci
FSH	Follicle-stimulating hormone
PRL	Prolactin
RNA-Seq	RNA sequencing
OXT	Oxytocin-neurophysin
CHRDL1	Chordin-like protein 1
GH	Growth hormone
PCA	Principal component analysis
qRT-PCR	Quantitative real-time PCR
CC	Cellular component
MF	Molecular function

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10233-1.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Author contributions
ZC, YZ, JC and FP designed the experiments. WZ, SZ and FW performed the data analysis of the transcriptome date. ZC, DW, and RM completed the manuscript writing. ZC and DW participated in the writing instruction and revision of the manuscript. All authors have read and approved the manuscript.

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Data availability
The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2021) in National Genomics Data Center (Nucleic Acids Res 2022), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA012981) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Declarations

Ethics approval and consent to participate
The animal protocol was approved by the Animal Ethics Committee of the Qiannan Normal University for Nationalities, and in compliance with ARRIVE guidelines 2.0.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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