Integration of transcriptome sequencing and whole genome resequencing reveal candidate genes in egg production of upright and pendulous-comb chickens

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ABSTRACT Egg production performance plays an important role in the poultry industry across the world. Previous studies have shown a great difference in egg production performance between pendulous-comb (**PC**) and upright-comb (**UC**) chickens. However, there are no reports to identify potential candidate genes for egg production in PC and UC chickens. In the present study, 1,606 laying chickens were raised, and the egg laid by individual chicken was collected for 100 d. Moreover, the expression level of estrogen and progesterone hormones was measured at the start-laying and peak-laying periods of hens. Besides, 4 PC and 4 UC chickens were selected at 217 d of age to perform transcriptome sequencing (**RNA-seq**) and whole genome resequencing (**WGS**) to screen the potential candidate genes of egg production. The results showed that PC chicken demonstrated better egg production performance ($P < 0.05$) and higher estrogen and progesterone hormone expression levels than UC chicken ($P < 0.05$). RNA-seq analysis showed that 341 upregulated and 1,036 downregulated differentially expressed genes (**DEGs**) were identified in the ovary tissues of PC and UC chickens. These DEGs were mainly enriched in protein-related, lipid-related, and nucleic acids-related biological processes including ribosome, peptide biosynthetic process, lipid transport terms, and catalytic activity acting on RNA which can significantly affect egg production in chicken. The enrichment results of WGS analysis were consistent with RNA-seq. Further, joint analysis of WGS and RNA-seq data was utilized to screen 30 genes and *CAMK1D, CLSTN2, MAST2, PIK3C2G, TBC1D1, STK3, ADGRB3,* and *PPARGC1A* were identified as potential candidate genes for egg production in PC and UC chickens. In summary, our study provides a wealth of information for a better understanding of the genetic and molecular mechanism for the future breeding of PC and UC chickens for egg production.

Key words: transcriptome sequencing, whole genome resequencing, upright and pendulous-comb, egg production performance

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INTRODUCTION

Egg production plays an important role in human food resources together with meat and milk (Gautron et al., 2022). However, egg production is a quantitative trait caused by many genes of small effect and its heritability value is extremely low (Biscarini et al., 2010; Goto and Tsudzuki, 2017). The ovary is the endocrine organ of the hen reproductive system and estrogen (**E**) or progesterone (**P**) is secret from the ovary and affects the egg production of chicken (Leszczynski et al., 1985; Tian et al., 2018). The laying hens are raised all over the world and how to further improve the egg production rate has been a major research interest for farmers.

The comb is not only a symbolic skin derivative of chicken but also one of the traits with multiple phenotypic variations. The phenotypes of the chicken comb can be mainly divided into the single, rose, pea, walnut comb, and duplex comb (Wright et al., 2009; Imsland et al., 2012; Dorshorst et al., 2015). Interestingly, multiple kinds of research have proved that phenotypes of the

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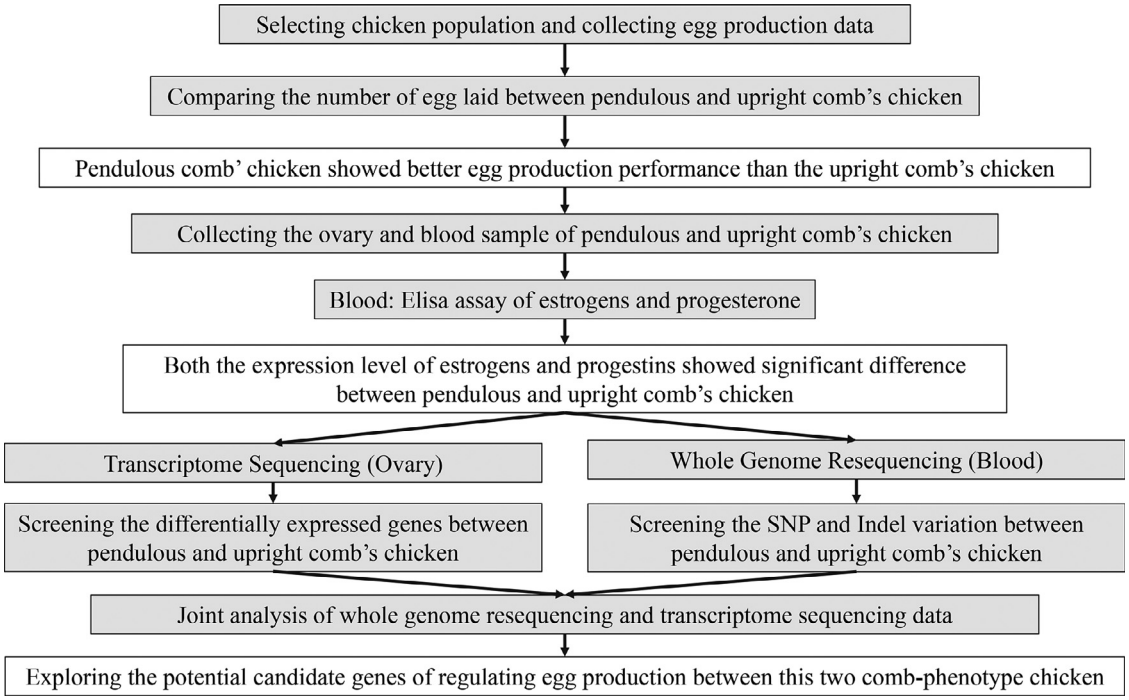

Figure 1. The experimental flow of this study. The experiment in this study can be divided into 4 parts, including the comparison of egg production and reproductive hormones expression level between PC and UC chickens, RNA-seq of PC and UC chicken's ovary tissue, WGS analysis of UC and PC chicken's genome, joint analysis of RNA-seq and WGS to screen potential candidate genes. Abbreviations: PC, pendulous-comb; UC, upright-comb; WGS, whole genome resequencing.

chicken comb are highly associated with chicken reproductive performance (Imsland et al., 2012; Navara et al., 2012). Especially in single-comb chicken, the candidate genes for regulating comb length, comb height, and comb weight were found related to follicular and gonadal development (Shen et al., 2016). Moreover, it was reported that the egg production rate of red-comb hen is significantly higher than the dark one, and the genes controlling the comb color are also associated with the ovarian function (Dong et al., 2019).

The pendulous comb is one of the common comb traits in single-comb chicken breeds especially in hens, which shows a comb flopping down along one side of the head (Guo et al., 2020). In a recent study, the egg production performance between pendulous-comb (**PC**) and upright-comb (**UC**) chickens is different (Wan et al., 2018). In indigenous chicken, the egg production of the PC chicken is significantly higher than that of the UC chicken. Basically, commercial laying hen breeds like white leghorn chicken possess pendulous-comb phenotype. However, few research have been performed to explore the underlying genetic and molecular mechanism of the egg production performance between PC and UC hens.

During the egg-laying period, the transcriptome pattern in reproduction-related tissue was significantly different between the high and low-production chicken. Recently, transcriptome analysis (**RNA-seq**) has been performed using the hypothalamus (Bello et al., 2021), pituitary (Wang and Ma, 2019), follicles (Chen et al., 2021), and ovary (Mu et al., 2021) to screen the candidate genes for egg production performance in different poultry species. Besides, with the rapid development of high-throughput sequencing technology, whole genome sequencing (**WGS**) has become a powerful method to detect the potential molecular markers related to quantitative trait (Li et al., 2021). Recent studies have performed WGS to successfully screen promising genes and SNP markers for egg production performance (Liu et al., 2019), chicken comb trait (Yang et al., 2021), and carcass and growth traits (Zhang et al., 2020) in chickens. The integration of RNA-seq and WGS can further screen the candidate genes for complex quantitative traits compared with a single sequencing technology (Xu et al., 2016; Bello et al., 2022).

In this study, we compared the egg production performance between PC and UC chicken and detected the secretion level of E and P hormones. Finally, WGS and RNA-seq using DNA samples and ovary tissues, respectively were performed to identify the potential candidate genes that might be regulating the egg production performance between PC and UC chickens. The experimental flow is shown in Figure 1.

MATERIALS AND METHODS

Experimental Animals

The F_1 generation (offspring produced by crossing Chinese indigenous spotted chicken and Chinese indigenous yellow chicken) containing 1,606 individual indigenous chickens were used for this study. All blood, serum, and ovary tissue samples were collected adhering strictly to the requirements of the Institutional Animal Care and Use Committee of South China Agricultural University (Approval number: SCAU#2021F074). Utmost

efforts were made to minimize the number of animals used with little or no suffering.

Experimental Sample Preparation

The experimental chickens were raised in floor pens and were subjected to a 12 h:12 h (light:dark cycle) until 147 d of age (pre-laying period). After that, chickens were divided into 2 groups according to their comb types (PC and UC) and transferred into individual cages with the same feeding and management conditions (fed with commercial corn-soybean-based diets).and the lighting condition was changed by adding 1 h of light per week until reaching 16 h:8 h (light:dark cycle).

The number of eggs laid for 100 d was measured from 175 to 275 d of age. The serum of 22 PC chickens and 22 UC chickens was randomly collected at the start-laying period (25 wk of age) and peak-laying period (31 wk of age). At 217 d of age (peak-laying period), 4 PC and 4 UC chickens with similar body weight, shank length, and shank girth were selected for collection of blood samples and ovary tissues with sterile scissors and tweezers. We collected the medullary layer of the ovary tissue after removing the follicles and fascia. Serum and blood samples were stored at −20°C and ovary tissue samples were stored at −80°C.

ELISA Assay

Elisa assay was performed to measure the expression level of E and P hormones using a Chicken Estrogen ELISA Kit and Chicken Progesterone ELISA Kit (mlbio, Shanghai, China) according to the manufacturer's protocol.

Genomic DNA Extraction, RNA Extraction, cDNA Synthesis, and qPCR Assay

The genomic DNA of the blood samples was extracted using NRBC Blood DNA Kit (Omega, Georgia, CA) according to the manufacturer's protocol. The total RNA of the 8 ovary tissue samples was extracted using RNAiso Plus (Takara, Kyoto, Japan) and the HiPure Universal RNA Mini Kit (Magen, Guangzhou, China) according to the manufacturer's protocol. cDNA was synthesized using HiScript III All-in-one RT SuperMix Perfect for qPCR (Vazyme, Nanjing, China) for reverse transcription. Primers were designed in NCBI Primer Design Tool. cDNA samples were subjected to ChamQ Universal SYBR qPCR Master Mix (Vazyme) according to the manufacturer's protocol. The $2^{-\Delta\Delta Ct}$ method and internal normalization were used to analyze quantification results (Livak and Schmittgen, 2001). The information on primers used for qPCR amplification was listed in Table S1.

Transcriptome Sequencing (RNA-seq)

The medullary layer of the ovarian tissue samples of 4 PC and 4 UC chickens were collected to extract total RNA using the RNAiso Plus (Takara) and the HiPure Universal RNA Mini Kit (Magen, Guangzhou, China) according to the manufacturer's protocol. RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA). The library preparations were sequenced on an Illumina Novaseq platform at Novogene Biotech Co. Ltd. (Beijing, China). The RNA-seq data reported in this study were archived in the GSA database with the accession number CRA008430 (https://ngdc.cncb.ac.cn/gsa/s/8GbEL8jK).

Identification of Differentially Expressed Genes and Alternative Splicing Analysis

Feature Counts v1.5.0-p3 (Liao et al., 2014) was used to count the reads numbers mapped to each gene. Afterward, fragments Per Kilobase of transcript sequence per Millions base pairs sequenced (**FPKM**) of each gene were calculated. The differential expression analysis between the PC and UC ovary samples was performed using the DESeq2 R package (1.20.0) (Love et al., 2014). Alternative Splicing (**AS**) is an important mechanism for regulating the expression of genes and the variable of protein. rMATS (4.1.0) software was used to analyze the AS event.

Whole Genome Resequencing

The genomic DNA of the 4 PC and 4 UC chickens' blood samples (consistent with RNA-seq individuals) extracted was used for whole genome resequencing (**WGS**). The library preparation was performed according to the standard Illumina protocol at Novogene Biotech Co. Ltd. (Beijing, China). The original image data generated by the sequencing machine were converted into sequence data via base calling (Illumina pipeline CASAVA v1.8.2) and then subjected to a quality control (**QC**) procedure to remove unusable reads. Sequencing reads were aligned to the reference genome using BWA with default parameters. The WGS data reported in this study were archived in the GSA database with the accession number CRA008936 (https://ngdc.cncb.ac.cn/gsa/s/NUsh30WR).

WGS Variant Detection and Annotation

The raw SNP/InDel sets were called by samtools with the parameters '-q 1 -C 50 -m 2 -F 0.002 -d 1000'. Then, we filtered these sets using the following criteria: 1) The mapping quality > 20; 2) The depth of the variate position > 4.

GO and KEGG Pathway Enrichment Analyses of DEGs

GO enrichment analysis of DEGs was conducted by the clusterProfiler (3.8.1) R package, in which gene length bias was corrected. Cluster Profiler (3.8.1) R package was used to test the statistical enrichment of DEG in KEGG pathways. GO terms and KEGG

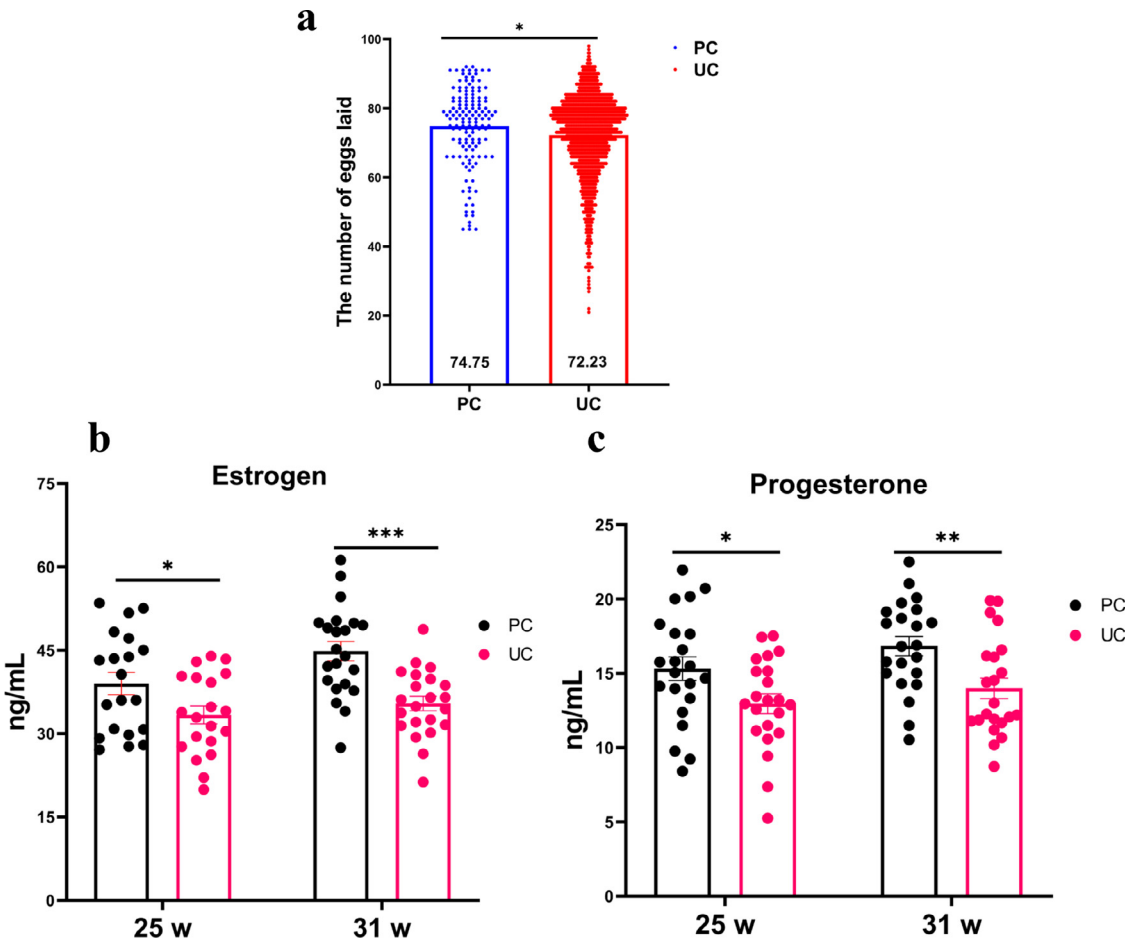

Figure 2. Comparison of the egg-laying number and the expression level of E and P hormone between PC and UC chicken. (A) PC chicken showed more egg-laying numbers than UC chicken. Besides, the expression level of (B) E and (C) P hormones in PC chicken was significantly higher than in UC chicken in both the start-laying period and the peak-laying period. Symbol "*", "**"and "***" indicated a significant difference at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. Abbreviations: PC, pendulous-comb; UC, upright-comb.

pathways with adjusted P-value < 0.05 were considered significantly enriched among DEGs.

Statistical Analysis

All experiments in this study were repeated 4 times at least to ensure repeatability and all data are expressed as means ± standard error of mean (**SEM**). An independent sample t test was used to compare differences between the 2 groups (i.e., PC and UC) and $P < 0.05$ was considered statistically significant between the groups. All statistical analyses were performed using SPSS 25.0 for Windows (SPSS, Inc., Chicago, IL). Symbols "*", "**" and "***" indicate a significant difference at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

RESULTS

PC Chicken Showed Better Egg Production and Higher Reproductive Hormone Level Than UC Chicken

To compare the egg production performance between PC and UC chickens, the number of eggs laid within 100 d was measured across the selected hen population. The results showed that PC chicken had more egg production than UC chicken ($P < 0.05$; Figure 2A). Besides, the Elisa assay was performed to compare the E and P hormone expression level of PC and UC chickens in the start-laying and the peak-laying periods. Interestingly, PC chicken showed significantly ($P < 0.05$) higher E and P expression levels than UC chicken in both periods (Figures 2B and 2C). The PC chicken demonstrated an extremely significant difference in both hormones' expression levels ($P < 0.01$ and $P < 0.001$), especially in the peak-laying period (Figures 2B and 2C). These results suggested that PC chicken had better egg production performance than UC chicken and the ovary might play an important role in this difference.

RNA-Seq Revealed a Significant Difference in the Gene Expression Pattern Between the Ovary Tissue of PC and UC chicken

Based on the results above, RNA-seq of ovary tissue was performed to reveal the gene expression pattern between PC and UC chicken. The information on

quality analyses of RNA-seq is presented in Table S2. In total, 14,472 genes were found in RNA-seq and 13,176 of them were co-expressed in PC and UC chicken (Figure 3A). Differentially expressed genes (DEGs) between PC and UC chicken's ovary were shown as a hierarchical clustering map (Figure S1). There are 341 upregulated and 1,036 downregulated DEGs (Figure 3B). The information on DEGs is listed in Table S3. Besides, some differentially alternative splicing events (**DAS**) were also detected, and skipped exon was a major part of DAS (Figure 3C). To affirm the reliability of the RNA-seq results, we randomly selected 9 upregulated and 5 downregulated DEGs for qPCR. We found that the result of qPCR was consistent with RNA-seq, which indicated the reliability of RNA-seq results (Figures 3D−3G).

Protein-Related, Lipid-Related, and Nucleic Acids-Related Terms Were Enriched in Differentially Expression Genes Between the Ovary Tissue of PC and UC chicken

To further explain the biochemical functions of the DEGs, the 1,377 DEGs (341 upregulated and 1,036 downregulated) were used to perform GO and KEGG enrichment analyses. GO terms were classified into the following three (3) types: biological process (**BP**), cellular component (**CC**), and molecular function (**MF**). In total, 56 GO terms were significantly enriched and the top 10 terms of BP, CC, and MF were shown in Figure 4A ($P < 0.05$). Among these enriched terms, we observed that protein-related, lipid-related, and nucleic acids-related terms were the most enriched. For example, translation, peptide biosynthetic process, amide biosynthetic process, peptide metabolic process and lipid transport terms in BP; cytoplasm, ribosome, cytoplasmic part, and ribonucleoprotein complex terms in CC, and structural constituent of ribosome, peptidyl-prolyl cis-trans isomerase activity, hydrolase activity, acting on glycosyl bonds, catalytic activity, acting on RNA terms in MF. This suggested that protein-related, lipid-related, and nucleic acids-related biological processes might play some roles in egg production. The DEGs were significantly enriched in eight (8) KEGG pathways ($P < 0.05$) especially ribosome, glycosaminoglycan biosynthesis-keratan sulfate, RNA transport, aminoacyl-tRNA biosynthesis, and amino sugar and nucleotide sugar metabolism and peroxisome. This also suggests that the DEGs were related to the anabolism of proteins, nucleic acids, and lipids (Figure 4B).

WGS Analysis Identified Many SNP and InDel Sites Between PC and UC Chicken

To further screen the candidate genes causing the different egg production performance between PC and UC chicken, WGS analysis was performed to screen the SNP and InDel sites between PC and UC chicken. The information on quality analyses, mapping rate, and average sequence coverage are listed in Table S4 and S5. Compared with the reference genome (bGalGal1.mat.broiler.GRCg7b), many SNP and InDel sites were detected in all samples (Figures 5A and 5B). We further screened the candidate genes according to their number of SNP or InDel sites and we selected the top 5% genes with the highest number of SNP or InDel sites (Table S6 and S7). Besides, GO enrichment analyses were performed on the biological function of these candidate genes. The enrichment results were also consistent with GO enrichment of RNA-seq. The protein-related, lipid-related, and nucleic acids-related terms were also enriched in WGS (Figures 5C and 5D).

Joint Analysis of WGS and RNA-Seq to Explore the Candidate Genes of Egg Production Performance Between PC and UC Chicken

Based on the result shown above, we performed a joint analysis of WGS and RNA-seq data to further screen the candidate genes that might affect egg production performance between PC and UC chicken. We selected the final 30 candidate genes from the intersection of the top 5% SNP sites enriched genes in WGS, the top 5% InDel sites enriched genes in WGS, and the DEGs in RNA-seq (Figure 6). The detailed information on these candidate genes was listed in Table S8. Considering the significant levels of the DEGs, SNP, and InDel sites number, GO and KEGG pathway results and literature reviews (Table 1), *CAMK1D, CLSTN2, MAST2, PIK3C2G, TBC1D1, STK3, ADGRB3*, and *PPARGC1A* were selected as potential candidate genes responsible for egg production performance of PC and UC chicken (Table 2).

DISCUSSION

Egg production performance is one of the most important reproductive performances of hens which plays an important role in the poultry industry (Awada et al., 2021). Egg production trait is a low-heritability trait that is difficult to improve by direct selection (Wolc et al., 2011). The phenotype of single comb in chicken is one of the high-heritability traits which has been proven to be highly associated with chicken reproductive performance especially egg production (Grimes et al., 1991; Eitan et al., 1998; Wan et al., 2018; Dong et al., 2019). PC is a common phenotype of single comb in chicken, especially in hens. Nevertheless, there are only a few studies on its relationship with chicken egg production performance. In the present study, we compared the egg production of PC and UC hens using the egg-laying data of 1,606 individuals recorded for a total of 100 d and discovered that the egg production performance was significantly higher in PC chicken than the UC counterpart. Moreover, we measured the expression level of E and P hormones in 2 key periods of egg production: the start-

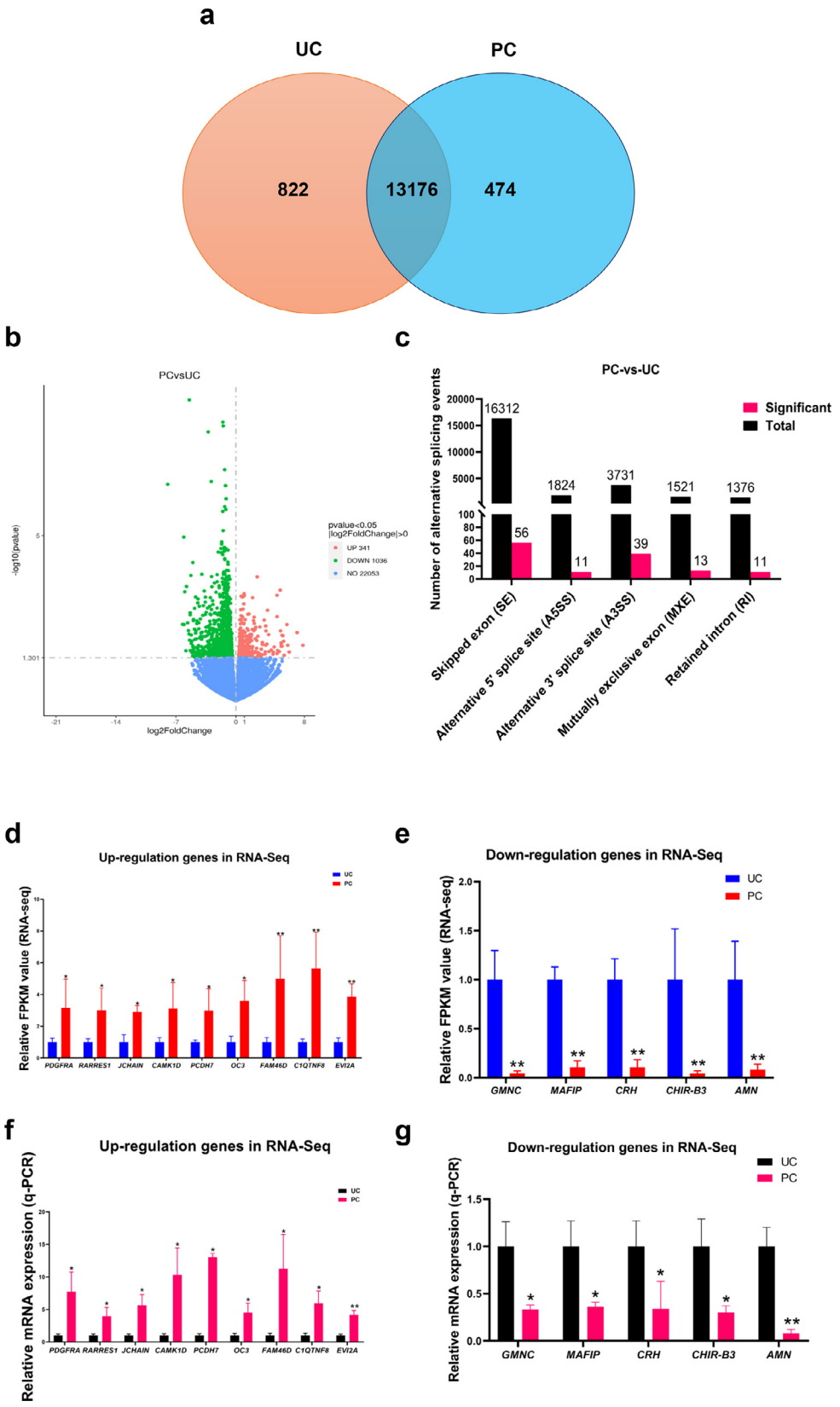

Figure 3. Transcriptome sequencing analysis of PC and UC chicken's ovary tissue. (A) 14,472 genes were detected in total and 13,176 of them were co-expressed in PC and UC chicken's ovary tissue. (B) The volcano plots a map of all DEGs between PC and UC chicken's ovary. Red dots represent significantly upregulated genes and green dots represent significantly downregulated genes (C) The number of alternative splicing events detected in transcriptome sequencing. Randomly selecting and showing the relative FPKM value of (D) 9 upregulated DEGs and (E) 5 downregulated DEGs in RNA-seq. The relative expression level of (F) 9 upregulated genes and (G) 5 downregulated genes were validated by qPCR. Symbol "*" and "**" indicated a significant difference at $P < 0.05$ and $P < 0.01$, respectively. Abbreviations: DEGs, differentially expressed genes; PC, pendulous-comb; UC, upright-comb.

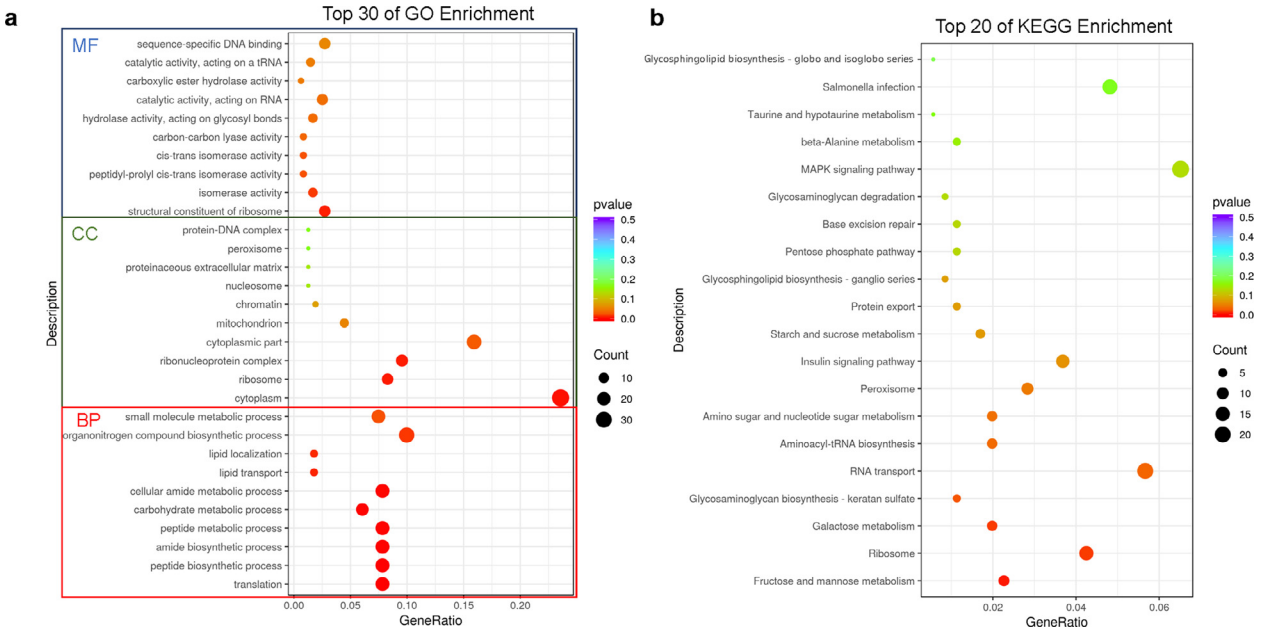

Figure 4. The GO and KEGG enrichment analysis of DEGs in transcriptome sequencing analysis. (A) GO enrichment analysis of DEGs in molecular function, cellular component, and biological process. (B) KEGG pathway enrichment analysis of enriched DEGs. Abbreviations: DEGs, differentially expressed genes; GO, Gene Ontology.

laying period and the peak-laying period, and the expression level of E and P hormones was also highly expressed in PC chicken compared with UC chicken. E and P hormones can significantly affect follicular growth (Liu et al., 2004). Although, the expression level of hormones is affected by a variety of endogenous and exogenous factors such as diet, sampling time, method of sample handling, storing, etc (Constantin et al., 2022). Therefore, evaluation of egg production by the expression level of hormones might not be sufficient, but there is no doubt that the difference in hormone expression level could be one of the major factors that affect the egg production performance between PC and UC chickens.

To further explore the gene markers of egg production performance, RNA-seq of ovary tissue and WGS analysis were performed between PC and UC chickens.

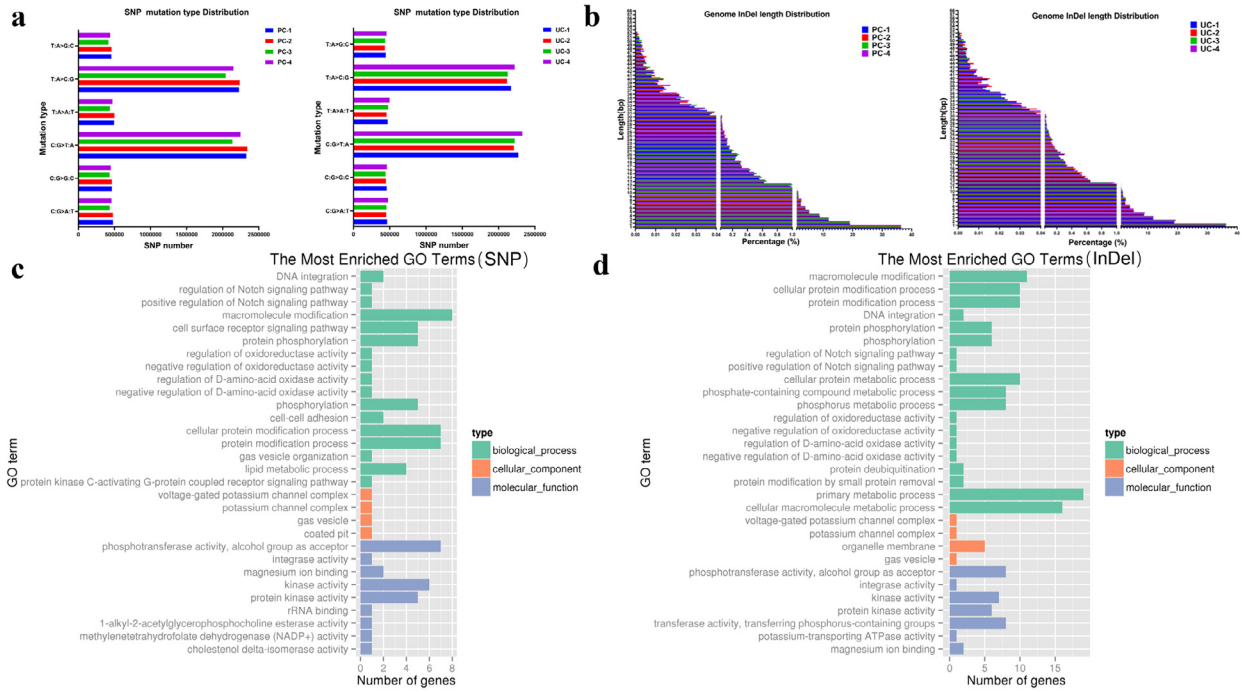

Figure 5. The WGS analysis of PC and UC chicken's genome. Both groups used 4 biological replicates, and the number of (A) SNP mutation type and (B) the percentage of InDel length distribution of each biological replicate was shown. GO enrichment analysis of (C) the top 5% SNP sites enriched genes and (D) the top 5% InDel sites enriched genes. The green, orange, and blue columns represented biological processes, cellular components and molecular function, respectively. Abbreviations: PC, pendulous-comb; UC, upright-comb.

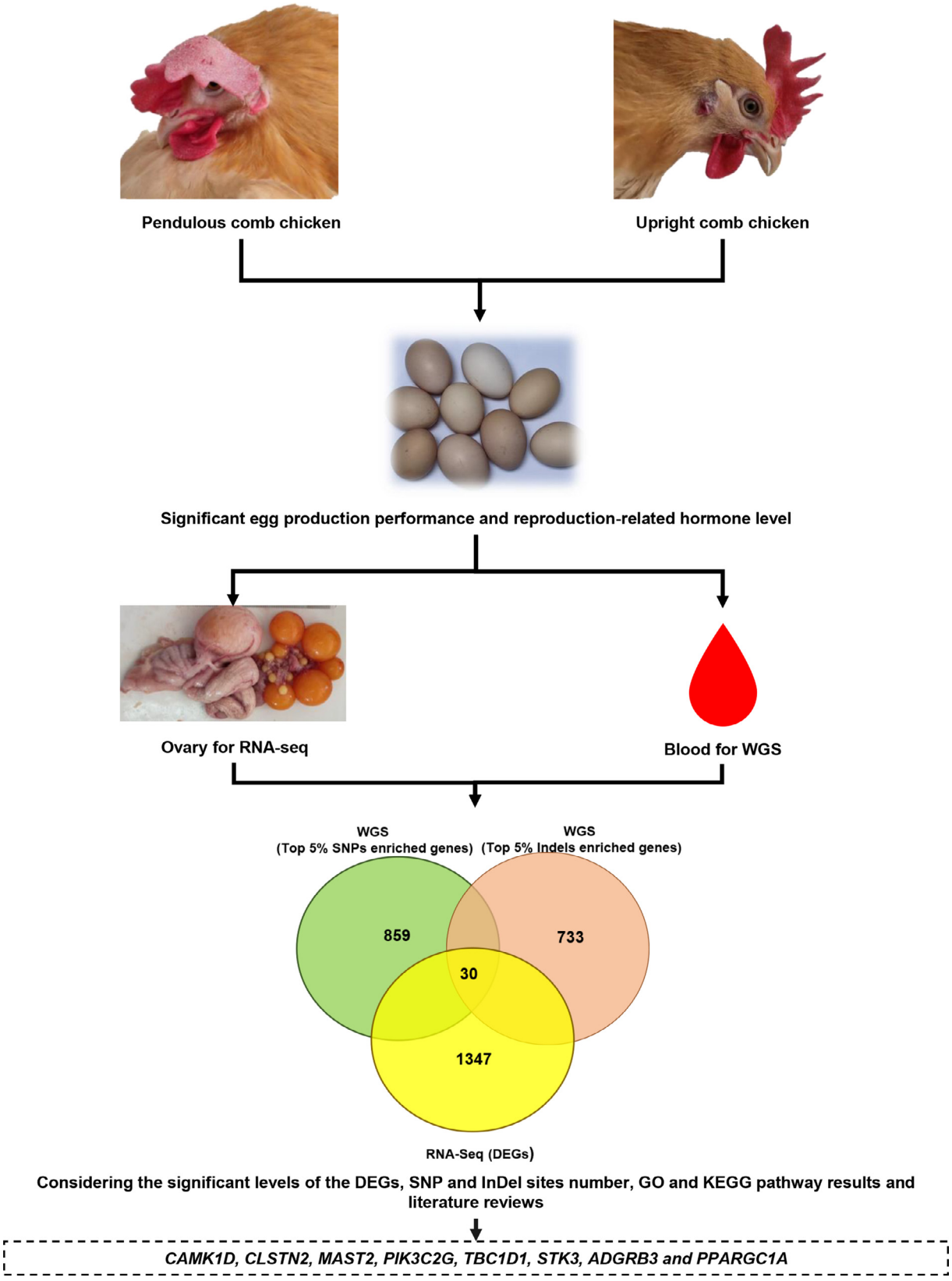

Figure 6. The flow of screening the potential candidate genes for egg production performance in PC and UC chicken. Joint analysis of WGS and RNA-seq data to further screen 30 candidate genes of egg production performance between PC and UC chicken. Considering the significant levels of the DEGs, SNP and InDel sites number, GO and KEGG pathway results and literature reviews, we selected 8 genes as the potential candidate genes for egg production performance considering the significant levels of the DEGs, SNP, and InDel sites number, GO and KEGG pathway results and literature reviews. Abbreviations: DEGs, differentially expressed genes; GO, Gene Ontology; PC, pendulous-comb; UC, upright-comb; WGS, whole genome resequencing.

Table 1. Functions and roles of candidate genes that have been reported.

Gene	Description	Functions	Relevant roles in literature reports (Literature reviews)
CAMK1D	Calcium/calmodulin-dependent protein kinase ID	Calcium-related genes	An important role in type 2 diabetes (Xue et al., 2018) A key modulator of tumor intrinsic immune resistance (Volpin et al., 2020)
CLSTN2	Calsyntenin 2	Calcium-related genes	The candidate gene affecting prolificacy in goats (Wijayanti et al., 2022) The gene associated with litter size in Pelibuey sheep (Hernández-Montiel et al., 2020)
MAST2	Microtubule-associated serine/threonine kinase 2	Egg production	The gene associated with a negative genetic correlation observed between growth and egg production performance in the female broiler (Tarsani et al., 2021)
PIK3C2G	Phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 gamma	Egg production	The homozygotes of advantageous alleles affect the eggshell quality (Sun et al., 2015)
TBC1D1	TBC1 domain family member 1	Follicular development	Affecting the dominant follicle selection and development (Ireland et al., 2009) The gene associated with dominant follicle selection and development (Zielak et al., 2007)
STK3	Serine/threonine kinase 3	Female reproductive system	Regulating and expressing in uterine endometrium during the estrous cycle (Moon et al., 2019) Indicated as one of the key candidate genes for litter size performance in goat (E et al., 2019)
ADGRB3	Adhesion G protein-coupled receptor B3	Ovary-related disease	Closely connected with the clinical manifestations of Uterine Corpus Endometrial Carcinoma patients (Lei et al., 2022)
PPARGC1A	PPARG coactivator 1 alpha	Ovary-related disease	The polymorphism loci are associated with polycystic ovary syndrome (San-Millán and Escobar-Morreale, 2010) Significant correlation with familial breast cancer (Wirtenberger et al., 2006)

Considering the results of RNA-seq, 1,377 DEGs including 341 upregulated genes and 1,036 downregulated genes were found in the ovary of PC compared with UC chickens. GO and KEGG enrichment analysis of DEGs significantly enriched 56 significant GO terms and 8 KEGG pathways which mainly were related to the anabolism of proteins, lipids and nucleic acids such as the ribosome, peptide biosynthetic process, lipid transport terms, and catalytic activity acting on RNA. Ribosome and peptide biosynthetic processes were important parts of the protein synthesis process. During the follicular development, numerous biological processes occurred including a series of protein synthetic (Zhou et al., 2020; Li et al., 2022). Many proteins such as bone morphogenetic protein 4 (Yao et al., 2020), and transcription factor CTIP2 (Bhattacharya et al., 2015) play important roles in chicken follicular development. The ovary is both an endocrine gland and a reproductive organ and it can secrete steroid hormones E and P to regulate follicular development (Yu et al., 2021). The DEGs enriched in lipid transport termed might affect the transport of steroid hormones which leads to the differential expression of E and P between PC and UC chicken. RNA also plays some roles in chicken follicular development (Peng et al., 2019). In addition, maternal RNAs and proteins in the oocyte are important for the early development of precursor cells of the oocyte in chicken (Rengaraj et al., 2020; Rengaraj and Han, 2022). Based on these processes, it is obvious that the DEGs involved between the ovary tissues of PC and UC chickens might be associated with egg production performance.

In addition, WGS analysis demonstrated that a large number of SNP and InDel sites were called between PC and UC chicken. The results of enrichment analysis of the top 5% SNP or InDel enriched genes showed that protein-related, lipid-related, and nucleic acids-related terms were enriched, which were consistent with RNA-seq. Summarily, both RNA-seq and WGS analysis suggested that the gene expression pattern and genome variation can be one of the

Table 2. Candidate genes for egg production performance between upright and pendulous comb chicken.

						SNP/InDel number		
Gene	Expression level (PC)	Expression level (UC)	log2FC	*P*-value	Expression level	PC_only	UC_only	PC_and_UC
CAMK1D	2423.30	781.25	1.6332	0.0448	Upregulated	472/31	570/47	783/38
CLSTN2	930.39	490.61	0.9231	0.0047	Upregulated	179/34	625/69	812/60
MAST2	2095.36	1271.79	0.7202	0.0371	Upregulated	160/11	423/27	590/32
PIK3C2G	514.14	323.23	0.6695	0.0487	Upregulated	392/31	222/23	643/43
TBC1D1	1858.04	1335.71	0.4761	0.0278	Upregulated	102/11	114/18	700/52
STK3	523.80	394.33	0.4099	0.0236	Upregulated	276/33	265/19	611/35
ADGRB3	23.98	48.20	−1.0035	0.0453	Downregulated	335/39	268/30	633/47
PPARGC1A	31.59	78.90	−1.3144	0.0211	Downregulated	311/25	477/43	857/55

factors that caused the difference in egg production performance between PC and UC chicken observed in this study.

Finally, we considered the intersection of the top 5% SNP and InDel enriched genes in WGS and DEGs in RNA-seq to screen the candidate genes that might affect egg production. Thirty genes were screened and listed in Table S8. Calcium is necessary for the formation process of egg production and it can significantly affect egg production performance (Wang et al., 2014, 2021; Dijkslag et al., 2021). In previous studies, it has been reported that both *CAMK1D* (Calcium/calmodulin-dependent protein kinase ID) and *CLSTN2* (Calsyntenin 2) are calcium-related genes. *CAMK1D* plays an important role in type 2 diabetes which might affect the secretion of steroid hormones that play key roles in egg production (Xue et al., 2018; Volpin et al., 2020; Wittert et al., 2021). *CLSTN2* has been identified as an important candidate gene for sheep prolificacy and an InDel in the *CLSTN2* gene sequence was highly associated with prolificacy in goats which can serve as a biomarker in marker-assisted selection (**MAS**) (Hernández-Montiel et al., 2020; Wijayanti et al., 2022). *MAST2* is microtubule-associated serine/threonine kinase 2 which was associated with a negative genetic correlation observed between growth and egg production performance in the female broiler (Tarsani et al., 2021). In a genome-wide association study about screening candidate genes for eggshell quality, the homozygotes of advantageous alleles of *PIK3C2G* genes had a better eggshell quality partly counteracting the negative effect of the aging process in chicken (Sun et al., 2015). *TBC1D1* was highly related to follicular development in cattle and it can affect the dominant follicle selection and development, follicular function, and oocyte quality (Zielak et al., 2007; Ireland et al., 2009). *STK3* is serine/threonine kinase 3 and plays an important role in the female reproductive system. Previous studies have shown that *STK3* responded to E and P hormones in the mouse uterine epithelium (Moon et al., 2019, 2022). Moreover, *STK3* was indicated as one of the key candidate genes for litter size performance in goats (E et al., 2019). *ADGRB3* and *PPARGC1A* were both associated with ovary-related disease and might play some roles in follicular growth (Wirtenberger et al., 2006; San-Millán and Escobar-Morreale, 2010; Lei et al., 2022). Considering the GO terms, DEGs, and literature reviews, we selected *CAMK1D, CLSTN2, MAST2, PIK3C2G, TBC1D1, STK3, ADGRB3,* and *PPARGC1A* genes as the candidate genes that might be responsible for differences in egg production performance between PC and UC chickens.

CONCLUSIONS

Conclusively, this is the first investigation to screen the candidate genes that might be responsible for differences in egg production performance between PC and UC chickens using RNA-seq (ovary-tissue) and WGS (DNA samples). Our study demonstrated that egg production performance and reproductive hormones were significantly different in PC and UC chickens. Furthermore, RNA-seq and WGS analysis revealed a significant differences in the gene expression pattern and a large number of SNP and InDel variations between PC and UC chickens. GO and KEGG analysis of DEGs and SNP or InDel enriched genes were mainly enriched in protein-related, lipid-related, and nucleic acids-related terms. Further, a joint analysis of WGS and RNA-seq was used to screen eight (8) candidate genes that could affect egg production performance between PC and UC chickens. These results provide important insight into the potential candidate genes that could influence the differences in egg production performances between PC and UC chicken, which lays a foundation for the construction of a MAS of egg production traits in chickens.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2023.102504.

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