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CCNA1 Gene Cloning, Tissue Expression and Its Polymorphic Association with Egg Production Traits in Ducks

Yifu Rao[1], Ai Liu[1], Wei Li [2], Jiaoyi Yang [1], Fuyou Liao [1], Binnong Yao[1], Shenglin Yang[1,3],* , Boonanuntan Surintorn [3]

1Key Laboratory of Animal Genetics, Breeding and Reproduction in the Plateau Mountainous Region, Ministry of Education, Guizhou University, Guiyang, 550025, China;

2Station of Livestock and Poultry Genetic Resources Management of Guizhou Province, Guiyang,550002, China；

3Guiyang Muren Agricultural Science and Technology Development Co. Ltd., Guiyang, 550600, Guiyang, Guizhou, China；

3School of Animal Production and Innovation, Suranaree University of Technology， Nakhon Ratchasima, 30000, Thailand;

ABSTRACT

To explore the polymorphisms of CCNA1 gene and their association with the egg production traits in ducks, the real-time PCR (RT-PCR) was used to detect the CCNA1 gene expression differences in different tissues and identify SNP sites by the DNAStar software MegAlign program combined with the sequencing peak map, to find the association of egg production traits with the polymorphic locus and haplotype combinations. The results showed that the mRNA expression level of the CCNA1 gene was the highest in the ovarian tissue. Six SNP sites, including g.1223 A>G, g.1600 T>C, g.1874 T>C, g.1901 C>T, g.1926 T>C and g.1931 G>A affected the egg production traits in ducks were detected. Association analysis showed that individuals with haplotype H3H4 had the highest DDP, while H2H2 individuals exhibited the highest AEW. The results suggested that the CCNA1 gene may be used as a candidate molecular marker to improve egg production traits in ducks. This study provides a scientific basis for further exploring the role and molecular mechanism of CCNA1 in the reproductive performance of ducks.

Keywords: CCNA1 gene; gene cloning; polymorphism; egg production traits; association analysis

INTRODUCTION

The low egg-production rate of ducks seriously affects the economic efficiency of their breeding industry. Therefore, elucidating the molecular genetic mechanisms underlying this physiological process has become a key issue to improve egg-production performance. The role of random mutations within introns in gene function has garnered increasing attention in recent years, with extensive research focusing on the influence of gene sequences. A substantial body of evidence indicates that intron mutations are related to disease phenotypes, growth, and reproductive traits in livestock and poultry. The elucidation and characterization of genes implicated in the reproductive biology of poultry are crucial for the enhancement of their reproductive performance (Gu et al., 2022). Although the reproductive performance of ducks has been gradually improved by conventional breeding methods, the rate of significant improvement has always been slow and, unlike mammals, the egg-laying mechanism of ducks has rarely been reported. Notably, follicle development is a key factor that directly affects egg-production performance (Li et al., 2021), and oocyte maturation is an important process in follicle development. Among them, the cell cycle regulatory protein A1 (Cyclin A1, *CCNA1*) has an important relationship with follicle maturation and genesis (Garg et al., 2017).

CCNA1 is a member of the highly conserved type A family of cell cycle proteins and plays an important regulatory role in cell proliferation by binding to cell cycle regulators, regulating DNA synthesis and

promoting cell entry into division (Cao et al., 2019). Simultaneously, *CCNA1* plays a significant part in the regulation of the M phase of the oocyte meiotic cell cycle （Fuchimoto et al., 2001. Miftakhova et al. (2016) report that aberrant expression of *CCNA1* has been found in breast cancer, acute myeloid leukemia, ovarian cancer, prostate cancer and esophageal squamous cell carcinoma. In the white goose brooding stage, follicle number is reduced and *CCNA1* expression in follicles is significantly lower (Yu et al., 2016). In studies of sheep litter size， the expression levels of *CCNA1* may have a significant effect on litter size in Small-Tail Han sheep (La et al., 2019). Meanwhile, *CCNA1* has been demonstrated to facilitate meiotic recovery in female mice, but its mechanism of action in female mice remains unclear（Li et al., 2020）

At present, the studies on the *CCNA1* gene at home and abroad are mainly focused on human and mouse (Fu et al., 2021), and there are fewer studies on other animals. The production and maturation of ova are key determinants of egg production and quality in birds. It has been demonstrated that *CCNA1* gene has an important effect on ovulation in humans and mice (Fatimah et al., 2024； Nergis et al.,2025). Nevertheless, A few reports could be found on the association of *CCNA1* gene polymorphism with egg production traits in ducks. In view of this, the present study is intended to examine the expression levels of *CCNA1* gene in different tissues during the peak laying period in the duck. Meanwhile, we will screen *CCNA1* gene for SNP loci to analyze the correlation between these polymorphic loci of *CCNA1* gene and egg production traits in order to identify the relevant molecular markers for the improvement of egg production traits and to provide a molecular basis for the selection and breeding of egg production traits in ducks.

MATERIALS AND METHODS

Ethics statement

All experimental procedures and sample collection were performed according to the Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China, revised in June 2004) and approved by the Animal Care and Use Committee of Animal Science College, Guizhou University, Guiyang, China (EAE-GZU-2021-E027).

Experimental animals and sample collection

A total of 68 (40-week-old) laying ducks were purchased from a company in Sansui County, Guizhou Province of China, for the feeding experiment. The experiment was conducted at a farm of Guizhou University. Birds were allocated to 3 groups; each group consisted of 17 ducks, which were reared in wired 3-level battery cages. The pre-test period lasted for 7 days, while the main test period lasted for 35 days. The nutritional levels of the experimental diets, including crude protein (15.24%), metabolic energy (11.46 MJ/kg), etc., were formulated according to the Criterion of Nutrient Requirements of Egg duck (SAC, GBT/41189-2021). During this period, the ducks were fed once a day at 07:00 am and 16:00, fed and watered freely, and the light of the duck house was kept at 16 h/d, with daylight supplementation at night until 22:00 pm. The ducks were reared according to the routine procedures of immunization and routine operating procedures. The egg production was recorded daily. At the end of experiment, the test ducks were fasted for 12 h, the necks were bled and executed, and the brain, heart, liver, ovary, spleen, lung, pectoral muscle, kidney, duodenum, leg muscle, myogastric, oviduct, glandular stomach, and uterus tissue samples of six egg-laying ducks were collected in RNA preservation solution and stored at -80 °C for backup. Five ml of blood samples were collected from the wing veins of each duck, stored in anticoagulation tubes and kept in a -20 °C refrigerator until use.

The egg production traits consisted of duck-day production (DDP), average egg weight (AEW), egg shape index (ESI), eggshell strength (ES), and eggshell thickness (ET). DDP was calculated as total eggs laid/number of laying ducks alive (68)/total number of days (35) in the period. AEW was calculated as the total weight of eggs/number of eggs. ESI, ES and ET were measured using the Egg Analyzer (model EA-01, ORKA Co., LTD, Israel).

RNA Extraction, cDNA Synthesis, and RT-PCR

Total RNA was extracted from each tissue sample of female ducks by Trizol method. The cDNA was synthesized by using the Thermo Scientific RevertAid Firt Strad cDNA Synthesis Kit K1621 kit (Thermo Fisher Scientific, Beijing, China) reverse transcription and stored at -20 ℃. The primers were designed using Primer Premier 5.0 software according to the gene sequence of *CCNA1* (registration number: XM_027470501.2) published in the GeneBank. The information of primer pairs was F-AAGAAGAAGACATCCCACCAG and R-CAGATCACGTCATCGCACA. The real-time fluorescence relative quantification of gene expression in each tissue sample was performed by SYBR Green I. Ultra SYBR Mixture SYBR Green I fluorescent dye was purchased from Beijing Kangwei Reagent Biotechnology Co. The reaction system was 50 μL: 2×Ultra SYBR Mixture 25 μL, Forward Primer 1 μL, Reverse Primer 1 μL, cDNA 2 μL, and ddH_2O supplemented to 50 μL. The PCR reactions were performed with the duck internal reference gene β-actin as the reference, and three replicates were made for each tissue sample and reference gene. The PCR amplification procedure was performed as follows: pre-denaturation at 94℃ for 5 min, followed by 39 cycles of denaturation for 30 s at 94℃, 65℃ for 5 s during annealing, extension for 50 s at 95 ℃.

PCR Amplification and DNA Sequencing

Blood samples were taken from the wings of 68 laying ducks into a syringe containing 2% EDTA, used as an anticoagulant and stored at -80℃ for further experiment. Genomic DNA was isolated from each duck's whole blood using Tiangen's blood DNA extraction kit (Beijing Tiangen). The primer pairs were designed using the online version of Primer 3.0 (http://primer3.ut.ee/) and presented as follows: F-AACCTGCTCTGTGCTACACT and R-GTTGTGCGTCACCTAGACAC，F-TCCAGTGTTCACCTACTGCTG and R-CAGCCCACCACATCCCAAATA. PCR was performed in a total volume of 20 μL， which included 8.0 μL of PCR Master Mix, 9 μL of RNase-Free Water, 1.0 μL (10 mol/L) of forward and reverse primers each, and 1.0 μL of genomic DNA. The reaction conditions were as follows: predenaturation at 95℃ for 6 min, and then denaturation at 94℃ for 30 s, annealing for 56℃ for 50 s, extension at 72℃ for 45 s, a total 35 cycles, and a final extension at 72℃ for 5 min. The reaction system was stored at 4 ℃ (Lei et al., 2008). Then, the amplified samples of the *CCNA1* gene were sent to Shanghai Biotechnology Co., Ltd. for sequencing.

Genotyping, statistical and association analyses

The following parameters were calculated based on all SNPs loci: Genotype frequency, allele frequency, and haplotype frequency. Genotype distribution chi-square value (χ^2), D' value and γ^2 value of linkage disequilibrium were calculated using SHEsis online software (http://analysis.bio-x.cn/). The observed heterozygosity (He), effective allele number (Ne) and polymorphic information content (PIC) were calculated manually. Generalized linear model (GLM) in SPSS18.0 software was used to analyze the correlation between genotypes or double haplotypes of SNP loci and the measured trait indexes.

$$Model: Y_{ij} = \mu + G_{ij} + e_{ij}$$

Y_{ij} represents the egg-laying trait phenotype, μ represents the mean, *Gij* represents the genotype effect, and e_{ij} denotes the random residual. One-way ANOVA or unpaired samples t-test was performed on the individual egg-laying means of each genotype. The least significant difference method (LSD) for multiple comparisons, and the results are expressed as mean ± standard error.

RESULTS

***CCNA1* gene expression in different tissues**

As shown in Figure 1, *CCNA1* gene had the hightest expression level in the ovary, followed by in the liver and heart tissues, and the differences in mRNA levels among three tissues were highly significant (P<0.05), The expression of the *CCNA1* gene varies to different degrees in other tissues, but the differences in expression levels among these tissues were not significant.

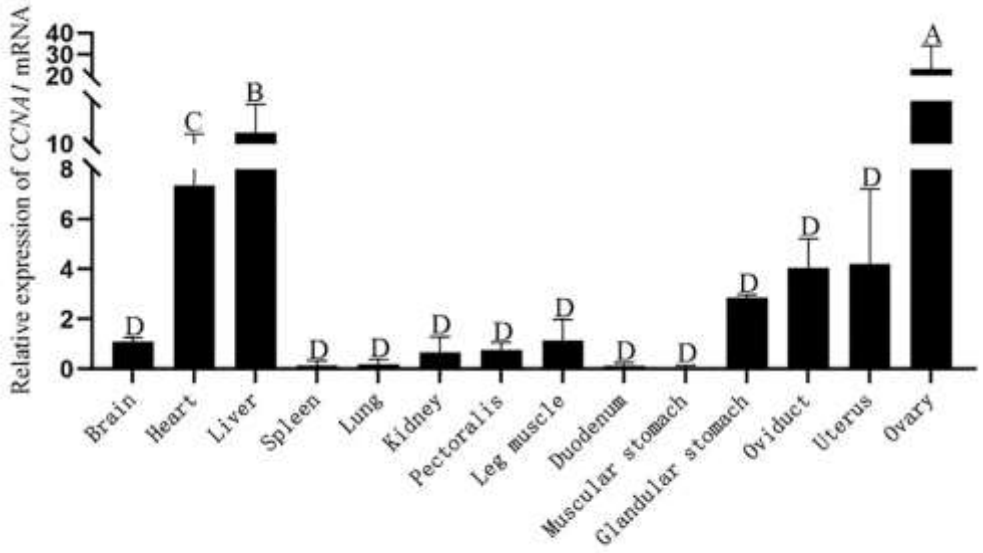

Figure 1. Relative expression levels of *CCNA1* gene in different tissues in ducks. The same letters in each group indicate non-significant differences(P＞0.05), different letters indicate highly significant differences (P<0.05).

Polymorphisms of the *CCNA1* gene

The PCR products were sequenced directly, and the sequence comparison results are shown in Figure 2. The results showed that a total of six SNPs variant loci were identified. g.1223 A＞G, g.1600 T＞C, g.1874 T＞C, g.1901 C＞T, g.1926 T＞C and g.1931 G＞A. g.1223 A＞G and g.1600 T＞C were in the CDS region of exon 3 of the *CCNA1* gene. The two SNP loci, g.1874 T＞C and g.1901 C＞T, were in the CDS region of exon 4 of the *CCNA1* gene, and g.1926 T＞C and g.1931 G＞A were in the intron 4 region of the *CCNA1* gene.

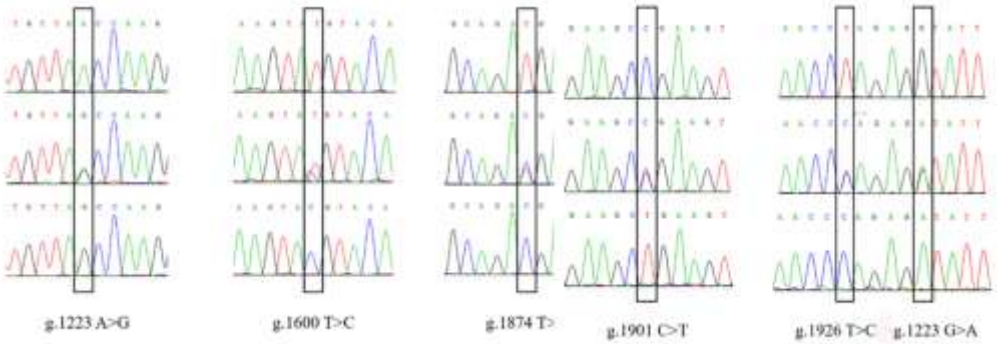

Figure 2. Sequence comparison peaks of *CCNA1* gene SNP loci.

The genetic parameters of the six SNP loci of the *CCNA1* gene were analyzed and the results are shown in Table 1. The number in the bracket represents the number of ducks with the corresponding genotype. All SNP loci were moderately polymorphic (0.25<PIC<0.5), of which three loci g.1600 T > C, g.1874 T > C and g.1926 T > C, were dominated by the allele T and genotype TT, g.1223 A > G locus allele A and genotype AA were dominant. Allele C of g.1901 C> T site and genotype CC were in the dominant position; the allele G at g.1931 G> A and genotype GG were in the dominant position. The Hardy-Weinberg equilibrium test showed that the genotype distribution of all six SNP loci did not deviate from equilibrium (P>0.05). Here, Ne denotes the effective number of alleles, PIC IS the polymorphism information content, χ^2 is the chi-square test value, which reflects the degree of difference between the observed values and the theoretical values.

Table 1. Genetic indices of SNP locus in the *CCNA1* gene

SNP loci	Genotype frequency	Allele frequency	Ne	PIC	χ^2			
g.1223	AA(22)	AG(31)	GG(15)	A(75)	G(61)			
A > G	0.324	0.456	0.221	0.551	0.449	1.979	0.372	0.419
g.1600	TT(18)	TC(34)	CC(16)	T(70)	C(66)			
T > C	0.265	0.500	0.235	0.515	0.485	1.998	0.375	0.000
g.1874	TT(43)	CT(23)	CC(2)	T(109)	C(27)			
T > C	0.632	0.338	0.029	0.801	0.199	1.467	0.268	0.269
g.1901	CC(29)	CT(32)	TT(7)	C(90)	T(46)			
C > T	0.426	0.471	0.103	0.662	0.338	1.810	0.347	0.178
g.1926	TT(39)	CT(22)	CC(7)	T(100)	C(36)			
T > C	0.574	0.324	0.103	0.735	0.265	1.637	0.314	1.940
g.1931	GG(18)	AG(34)	AA(16)	G(70)	A(66)			
G > A	0.265	0.500	0.235	0.515	0.485	1.998	0.375	0.000

Note: PIC > 0.5 of high polymorphism; 0.5 > PIC > 0.25 of moderate polymorphism; PIC < 0.25 of low polymorphism; $\chi^2_{0.05(2)}$ =5.991.

Linkage disequilibrium analysis between *CCNA1* gene SNP loci

The results of the linkage disequilibrium analysis for the six SNP loci of the *CCNA1* gene are presented in Table 2., Only the D′and R2 values between the other loci of g.1223 A＞G do not satisfy |D′| > 0.8 and R2>0.33, and there was no strong linkage disequilibrium. g.1600 T＞C and g.1901 C＞T , g.1926 T > C and g.1931 G > A satisfy this condition and there was a strong chain imbalance. g.1901 C > T and g.1600 T > C, g.1874 T > C, g.1926 T > C and g.1931 G > A had a strong chain imbalance.

Table 2. Analysis of linkage disequilibrium between SNP loci of the *CCNA1* gene.

SNP loci	g.1223 A > G	g.1600 T > C	g.1874 T > C	g.1901 C > T	g.1926 T > C	g.1931 G > A
g.1223 A > G	-	0.468	1.000	0.195	0.012	0.468
g.1600 T > C	0.209	-	1.000	1.000	1.000	1.000
g.1874 T > C	0.305	0.263	-	1.000	0.441	1.000
g.1901 C > T	0.024	0.542	0.485	-	1.000	1.000
g.1926 T > C	0.000	0.382	0.134	0.704	-	1.000
g.1931 G > A	0.209	1.000	0.263	0.542	0.382	-

Haplotype compositions of the linkage regions

The haplotypes and haplotype combinations of 6 SNP loci of the *CCNA1* gene are shown in Table 3. The results showed that 6 haplotypes and 13 haplotype combinations were detected in the whole genome of the *CCNA1* gene. The haplotype H1 displayed the highest frequency, 0.412, and became a dominant haplotype, while haplotype H6 had the lowest frequency, 0.074, and presented the inferior haplotype. The haplotype

H1H4 was the dominant haplotype with the highest frequency 0.176, whereas H2H2, H3H4 and H5H6 were regarded as inferior haplotypes with the lowest frequency 0.029.

Table 3. Haplotype and haplotype combinations of *CCNA1* gene SNP loci.

Haplotypes	g.1223 A > G	g.1600 T > C	g.1874 T > C	g.1901 C > T	g.1926 T > C	g.1931 G > A	Haplotype frequency
H1(56)	A	T	T	C	T	G	0.412
H2(17)	G	C	C	T	C	A	0.125
H3(20)	G	C	T	C	T	A	0.147
H4(19)	A	C	T	T	C	A	0.140
H5(14)	G	T	T	C	T	G	0.103
H6(10)	G	C	C	T	T	A	0.074
H1H1(10)	AA	TT	TT	CC	TT	GG	0.147
H1H2(4)	AG	CT	CT	CT	CT	AG	0.059
H1H3(8)	AG	CT	TT	CC	TT	AG	0.118
H1H4(12)	AA	CT	TT	CT	CT	AG	0.176
H1H5(8)	AG	TT	TT	CC	TT	GG	0.118
H1H6(4)	AG	CT	CT	CT	TT	AG	0.059
H2H2(2)	GG	CC	CC	TT	CC	AA	0.029
H2H4(5)	AG	CC	CT	TT	CC	AA	0.074
H2H5(4)	GG	CT	CT	CT	CT	AG	0.059
H3H3(3)	GG	CC	TT	CC	TT	AA	0.044
H3H4(2)	AG	CC	TT	CT	CT	AA	0.029
H3H6(4)	GG	CC	CT	CT	TT	AA	0.059
H5H6(2)	GG	CT	CT	CT	TT	AG	0.029

Association of *CCNA1* gene SNP loci and haplotype combinations with egg production traits in ducks

Association analysis of *CCNA1* gene SNP loci with egg production traits is listed in Table 4. Each SNP locus generated three different genotypes, two of them had a higher DDP than the other among these three genotypes. The highest DDP was observed in the CT genotype of the g.1926 T > C locus. The TT genotype of g.1874 T＞C loci displayed the highest AEW(72.665) compared with the other genotypes and the other loci ($P<0.01$), while the CT genotype of g.1874 T＞C loci had the lowest AEW(62.398), which was similar to the CT genotype of g.1901 C＞T. The other genotypes at the other loci had similar AEW values ($P<0.05$). The higher ESI values were detected at AA of g.1223 A> G, CC of g.1600 T> C, CC of g.1901 C＞T, CC of g.1926 T＞C, and AA of g.1931 G> A compared to the genotypes of the other loci ($P<0.05$). Accordingly, the higher ES values were observed at AA and AG of g.1223 A> G, CC of g.1600 T> C, TT and CC of g.1874 T> C, TT and CC of g.1901 C> T, TT and CC of g.1926 T＞C, and AA of g.1931 compared with the other loci ($P<0.05$). However, no significant differences in ET values were detected among all genotypes and loci ($P＞0.05$).

Table 4. Association analysis of *CCNA1* gene SNP loci with egg production traits in ducks

SNP loci	Genotypes	DDP	AEW	ESI	ES	ET
g.1223	AA	39.77±18.63^a	65.90±4.34^b	1.37±0.06^a	47.16±6.76^a	0.33±0.03
A > G	AG	39.68±15.47^a	64.50±5.93^b	1.33±0.12^c	46.42±7.95^a	0.32±0.03
	GG	35.60±19.74^b	64.02±5.10^b	1.35±0.05^b	41.81±4.90^b	0.31±0.04
g.1600	TT	36.69±21.19^b	64.68±5.50^b	1.32±0.11^c	44.47±6.33^b	0.31±0.03
T > C	CT	39.41±15.62^a	64.52±5.09^b	1.35±0.09^b	44.24±6.01^b	0.32±0.03
	CC	39.56±17.56^a	65.62±5.56^b	1.37±0.04^a	49.33±8.68^a	0.33±0.04
g.1874	TT	31.53±16.08^c	72.67±3.37^a	1.34±0.04^c	46.36±3.15^a	0.32±0.01
T > C	CT	38.13±17.38^a	62.40±4.45^c	1.32±0.13^c	44.10±7.67^b	0.32±0.03
	CC	40.23±17.14^a	65.80±5.13^b	1.37±0.05^a	46.43±7.06^a	0.32±0.03
g.1901	CC	39.41±16.29^a	65.87±5.39^b	1.37±0.04^a	47.00±7.88^a	0.32±0.03
C > T	CT	40.09±18.12^a	63.73±4.68^c	1.34±0.10^b	43.84±6.40^b	0.32±0.03

	TT	30.43±18.07^{c}	65.74±6.87^{b}	1.29±0.16^{c}	48.27±6.67^{a}	0.32±0.03
g.1926	TT	30.43±18.07^{c}	65.74±6.87^{b}	1.29±0.16^{c}	48.27±6.67^{a}	0.32±0.03
T > C	CT	40.86±17.08^{a}	64.69±4.99^{b}	1.33±0.11^{c}	44.25±5.21^{b}	0.33±0.03
	CC	39.15±17.36^{a}	64.78±5.23^{b}	1.37±0.05^{a}	45.96±8.19^{a}	0.32±0.03
g.1931	GG	36.69±21.19^{b}	64.67±5.49^{b}	1.32±0.11^{c}	44.47±6.33^{b}	0.31±0.03
G > A	AG	39.41±15.62^{a}	64.52±5.09^{b}	1.35±0.09^{b}	44.24±6.01^{b}	0.32±0.03
	AA	39.56±17.56^{a}	65.62±5.56^{b}	1.37±0.04^{a}	49.33±8.68^{a}	0.33±0.04

Mean±SE values with different superscripts in the same column means significant difference (P<0.05); mean±SE values with the same superscripts or without superscripts in the same column means no significant difference (P＞0.05).

The association analysis of haplotype combinations of the *CCNA1* gene loci with egg production traits in the *CCNA1* gene is shown in Table 5. It can be seen from Table 5 that DDP of individuals of haplotype combination H3H4 was significantly higher than that of the other haplotype combinations (P<0.05), while H2H2 individuals exhibited the lowest DDP but had the highest AEW compared to other combinations (P<0.05), whereas the other haplotype combinations had moderate DDP. In regard to AEW, no significant differences were observed among most haplotype combinations except for the H1H6, H2H5, H3H6 and H5H6 haplotype combinations, which showed smaller AEW, while H2H2 showed the largest AEW.

Table 5. Association analysis haplotypes combinations with egg production traits in ducks

Haplotypes	DDP	AEW	ESI	ES	ET
H1H1(10)	41.20±19.51^{b}	66.34±3.90^{b}	1.37±0.04^{a}	49.76±7.82^{a}	0.34±0.03^{ab}
H1H2(4)	35.00±10.52^{c}	64.05±6.33^{bc}	1.22±0.21^{b}	40.03±3.69^{b}	0.32±0.01^{b}
H1H3(8)	37.13±16.28^{c}	66.58±5.94^{b}	1.38±0.05^{a}	44.51±3.12^{a}	0.30±0.02^{bc}
H1H4(12)	38.58±18.66^{c}	65.53±4.81^{bc}	1.37±0.07^{a}	44.99±5.08^{a}	0.33±0.03^{a}
H1H5(8)	37.50±15.85^{c}	64.72±7.34^{bc}	1.37±0.03^{a}	48.77±10.71^{a}	0.31±0.04^{ab}
H1H6(4)	47.00±10.52^{b}	61.38±3.86^{c}	1.36±0.06^{a}	48.33±11.81^{a}	0.32±0.02^{b}
H2H2(2)	16.00±0.00^{d}	72.67±3.37^{a}	1.34±0.04^{a}	46.36±3.15^{a}	0.32±0.01^{b}
H2H4(5)	36.20±18.55^{c}	62.97±5.86^{c}	1.27±0.19^{b}	49.04±7.85^{a}	0.32±0.04^{b}
H2H5(4)	43.00±16.13^{b}	61.99±4.83^{c}	1.35±0.02^{a}	44.87±6.24^{a}	0.34±0.05^{a}
H3H3(3)	44.67±10.26^{b}	65.46±4.23^{b}	1.35±0.06^{a}	39.62±1.52^{b}	0.30±0.01^{bc}
H3H4(2)	62.00±8.49^{a}	66.33±5.89^{b}	1.32±0.01^{a}	46.92±5.87^{a}	0.36±0.01^{a}
H3H6(4)	29.00±28.91^{d}	61.39±3.73^{c}	1.34±0.07^{a}	40.24±3.59^{b}	0.29±0.03^{c}
H5H6(2)	40.00±22.63^{b}	62.53±1.03^{c}	1.39±0.06^{a}	37.57±5.14^{b}	0.33±0.03^{a}

Mean±SE values with different superscripts in the same column mean significant difference (P<0.05); mean±SE values with the same superscripts or without superscripts in the same column mean no significant difference (P＞0.05).

The egg-shaped index ESI showed only a slight difference in the H1H2 individuals, while there was no significant overall difference among the individuals of the various haplotype combinations. The higher egg shell strength (ES) values were detected in haplotype H1H1 and H1H5 individuals compared with haplotype H1H2, H3H3, H3H6 and H5H6 (P<0.05). Egg shell thickness (ET) was highly significantly higher in the H3H4 individual than that in the other haplotype combinations (P<0.05).

DISSCUSION

In mammals, *CCNA1* appears to be stage-specific and is highly expressed in germ cells（Fuchimoto D et al., 2001）. Similar to the present study, a previous study of small-tailed Han sheep showed that *CCNA1* mRNA expression level was observed in the hypothalamic-pituitary-gonadal axis tissues, with the highest in the uterus and the second in the ovary. Nevertheless, there is a paucity of research on ducks (La et al., 2019). In the present study, the Real-time fluorescence quantitative PCR assay showed that the *CCNA1* gene was expressed in different degrees in the brain, heart, liver, glandular stomach, uterus, ovary, and other tissues in the duck. Among these expressions, the expression level of the *CCNA1* gene was the highest in the ovary, followed by liver and heart tissues, and the mRNA level of these three tissues was significantly

higher than that in other tissues (Figure 1). These results indicate that *CCNA1* may be an important regulator in the normal physiological processes of the ovary.

As mentioned earlier, the *CCNA1* gene is involved in the regulation of many diseases and cancers. The Cyclin A1 protein, encoded by the *CCNA1* gene, is an important cell cycle regulator, belonging to the MAPK/ERK signaling pathway directly involved with thyroid cancer. Thus, the *CCNA1* gene can be as a potential diagnostic marker in papillary thyroid cancer (Raissa et al., 2020). Xiao et al. (2024) reported that *CCNA1* inhibited osteoporosis by suppressing the transforming growth factor-beta (TGF-beta) pathway in osteoblasts. As we know, the ovary is a female reproductive organ, which is intimately associated with egg-laying characteristics and the ovarian function of poultry has a direct impact on egg production (Yang et al., 2019). The progesterone-mediated oocyte maturation pathway (progesterone-mediated oocyte maturation) is a potential pathway associated with egg production. The *CCNA1* gene plays a pivotal role in follicle maturation and ontogenesis within this pathway. However, there is a paucity of research exploring the genetic polymorphism of the *CCNA1* gene and its association with egg-laying traits in ducks.

Several candidate genes have been identified in ducks as being highly expressed in the ovary and that are associated with egg-laying traits. Among these are the growth hormone (GH) (Wu et al., 2014), prolactin (PRL) (Bai et al., 2019), insulin-like growth factor-2 (IGF-2) (Ye et al., 2017), melatonin receptor (MTNR) (Feng et al., 2018), ovoinhibitor (OIH) (Wu et al., 2018), and follicle-stimulating hormone receptor (FSHR) genes (Xu et al., 2017). To the author's knowledge, no research reports related to the polymorphism of the CCNA*1* gene and its association with egg production traits have been found so far. Here, we reported the genetic information of the six SNP loci of the *CCNA1* gene in the duck population (Table 1). In this study, six SNP loci were identified by direct sequencing for the *CCNA1* gene: g.1223 A > G, g.1600 T > C, g.1874 T > C, g.1901 C > T, g.1926 T > C and g.1931 G > A. The results showed that all six SNP loci were moderately polymorphic (0.25 < PIC < 0.5), the three loci g.1600 T > C, g.1874 T > C and g.1926 T > C were dominated by the allele T and genotype TT; g.1223 A > G locus allele A and genotype AA are dominant. Allele C of g.1901 C > T site and genotype CC were in the dominant position; the allele G at g.1931 G > A and genotype GG are in the dominant position. Hardy-Weinberg equilibrium test showed that the genotype distribution of all six SNP loci did not deviate from equilibrium (P>0.05). This result suggests that the *CCNA1* gene frequencies and genotype frequencies of the population remain stable and unchanged during the genetic process.

This study also identified 6 previously unknown haplotypes and 13 haplotype combinations. We are currently preparing to submit them to GenBank (Table 5). As molecular markers, numerous studies have shown that SNPs are increasingly valuable for relevant studies in poultry breeding, as they are associated with many important production traits in livestock (Qin et al., 2023; Zhang et al., 2023). Association analysis of *CCNA1* gene SNP loci with egg production traits was performed in this study. CT genotype of g.1926 T > C loci. and TT genotype of g.1874 T > C loci displayed the highest average egg weight (AEW), while AA of g.1223 A> G, CC of g.1600 T> C, CC of g.1901 C > T, CC of g.1926 T > C, and AA of g.1931 G> A had higher ESI values. We can consider these genotypes as genetic markers for selecting ducks for egg quality performance. Association analysis of the haplotype showed that the haplotype-SNP of *CCNA1* was significantly associated with DDP. Individuals with haplotype H3H4 had the highest DDP compared to the other haplotypes, while H2H2 individuals exhibited the lowest DDP but had the highest AEW. In the breeding process, we can use H3H4 as a marker for enhancing DDP, and H2H2 as a genetic marker for improving AEW. However, it is worth noting that these two traits are negatively correlated.

In conclusion, this study found that the mRNA expression level of the *CCNA1* gene was the highest in the ovarian tissue. Six SNP sites of the *CCNA1* gene affecting the production, egg weight and egg quality in ducks were detected. Association analysis suggested *CCNA1* may be a valuable molecular marker affecting reproductive traits in ducks. This marker will help to improve the selection efficiency of egg-production

performance. However, given the restricted number of laying ducks, further investigation with a larger cohort of diverse breeds is essential to more rigorously assess the association with *CCNA1* gene polymorphisms and reproductive traits. Furthermore, additional research on *CCNA1* gene polymorphism is required to enhance the egg-laying performance of laying ducks.

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CONFLICTS OF INTEREST

The authors do not have any conflicts of interest declared.

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