

Identification and Genetic Effect of Haplotypes in the Distal Promoter Region of Chicken *CCT6A* Gene Associated with Egg Production Traits

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The chaperonin containing TCP-1 complex protein 1 subunit zeta (*CCT6A*) is the only cytosolic chaperonin in eukaryotes assisting in the folding of cytoplasmic proteins. Previous study revealed that the mRNA expression of chicken *CCT6A* gene was remarkably elevated in the sexually mature ovaries. However, the mechanism underlying chicken *CCT6A* expression changes remains largely unknown. In this study, haplotypes caused by two single nucleotide polymorphisms (SNPs) of chicken *CCT6A* gene promoter (g.-2215 T>C and g.-1959 T>C) were identified and their associations with egg production traits as well as effects on gene expression were analyzed. Altogether four haplotypes including A (C⁻²²¹⁵-T⁻¹⁹⁵⁹), B (C⁻²²¹⁵-C⁻¹⁹⁵⁹), C (T⁻²²¹⁵-T⁻¹⁹⁵⁹) and D (T⁻²²¹⁵-C⁻¹⁹⁵⁹) were detected in all of the five chicken populations. Diplotypes AA, AD and DD were predominant in Xinyang brown hens, among which diplotype AD was associated with higher egg number at the age of 28 weeks old (E28) ($P < 0.05$). In addition, diplotype AD was also predominant in Xinyang brown and Hy-line brown chicken populations with high egg production; whereas in Wenchang and Shouguang chicken populations which are Chinese indigenous chicken breeds and relatively lower in egg production, diplotype AA was predominant. Compared with diplotypes AA and DD, the mRNA expression of *CCT6A* in diplotype AD birds is the highest in F1, F5, and POF1 follicles of Hy-line brown hens ($P < 0.05$). These results suggest that the two SNPs in chicken *CCT6A* promoter region are potential DNA marker for improving egg production trait.

Key words: chaperonin containing TCP-1 complex protein 1 subunit zeta, chicken, egg production traits, Haplotype, polymorphisms, promoter

J. Poult. Sci., 53: 111-117, 2016

Introduction

Chaperonin containing TCP-1 complex (CCT) plays a fundamental role in the folding of cytoplasmic proteins, i.e. actin, tubulin, cyclin E, myosin, transducin and the Von Hippel Lindau tumor suppressor (Sternlicht *et al.*, 1993; Satish *et al.*, 2010). It contains eight different homologous proteins, including alpha, beta, gamma, delta, epsilon, eta, theta and zeta (Rommelaere *et al.*, 1993; Kubota *et al.*, 1994, 1995; Hynes *et al.*, 1995). The zeta subunit of CCT encoded by *CCT6A* gene is the only cytosolic hetero-oligomeric molecular chaperone assisting in the folding of cytoplasmic proteins. Human *CCT6A* gene is expressed in ten human tu-

mor cell lines (Myung *et al.*, 2004) and is up-regulated in an endometrial adenocarcinoma cell line after treatment with megestrol acetate (Zhang and Lin, 2006). In mice, it was detected on the surface of spermatozoa (Dun *et al.*, 2011).

The chicken *CCT6A* gene is located on chromosome 19 and consists of 14 exons. Our previous studies on chicken *CCT6A* gene indicated that, compared with sexually immature Jining Bairi hens, the expression of *CCT6A* mRNA was remarkably elevated in the ovaries of sexually mature hens; its expression in ovary was significantly increased from 70-day-old to 300-day-old hens, and decreased dramatically to 500-day-old hens (Kang *et al.*, 2012). During follicle growth, chicken *CCT6A* mRNA expression was increased rapidly from pre-hierarchy follicles to F5 follicles and subsequently declined in pre-ovulatory and post-ovulatory follicles in Hy-line brown hens (Wei *et al.*, 2013), which implies that *CCT6A* is involved in follicle recruitment from pre-hierarchy follicles. We also found that the expression of chicken *CCT6A* in granulosa cells was regulated by pro-

Received: July 21, 2014, Accepted: October 10, 2015

Released Online Advance Publication: November 25, 2015

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gesterone and follicle-stimulating hormone and identified a progesterone response element in the distal promoter region of chicken *CCT6A* gene (Wei *et al.*, 2013).

Egg production traits, such as age at first egg (AFE), egg number, egg weight and interval between oviposition time, are among the most important economic traits in egg-laying poultry (Kim *et al.*, 2004). Up to date, polymorphisms in the chicken *CCT6A* gene and its association with egg production traits were not reported. Due to the fact that the temporal expression of chicken *CCT6A* is consistent with sexual maturation, follicle growth and ovary function, and that deletion from -2534 to -1549 of chicken *CCT6A* gene promoter region cause up-regulation in luciferase activity (Wei *et al.*, 2013), therefore, in this study, we set out to find polymorphisms in this promoter region of chicken *CCT6A* and further analyze their genetic effects on egg production traits and mRNA expression level.

Materials and Methods

Birds and Trait Measurements

Individuals were randomly sampled from two Chinese indigenous chicken breeds (66 Wenchang chickens, Hainan Province; 49 Shouguang chickens, Shandong Province), one synthetic broiler line (55 Luqin synthetic broiler lines, Shandong Province), one synthetic laying line (194 196-day-old Xinyang brown chickens established by crossing heterozygotes at the polymorphic sites, Shanghai Poultry Breeding co., Ltd) and one commercial egg-laying lines (42 Hy-line brown chickens, Shandong province) used for polymorphism analysis on chicken *CCT6A* gene promoter region. The Xinyang brown chickens were also used for trait-genotype association analysis. For Xinyang brown chickens, they were randomly sampled and housed individually in laying batteries with free access to feed and water and were exposed to a 16L:8D photoperiod. In addition, the laying pattern of each hen of Xinyang brown chickens was recorded daily, and egg production traits including age at first egg (AFE) and egg number at 28 weeks of age (E28) were collected. Procedures concerning chicken management and slaughter were approved by the Ethics Committee of Laboratory Animals at Shandong Agricultural University.

DNA Extraction and Identification of Nucleotide Changes

Sampling was performed by taking 1 mL blood per hen from wing vein into collection tubes containing heparin sodium and held on ice until delivered to the laboratory. The

genomic DNA was extracted from blood sample using DNA Extraction mini kit (Tiangen, Beijing, China) and stored in TE (pH 8.0) at -20°C . Primer set CCT6A-P as shown in Table 1 was designed with DNAMAN software version 7.0 according to the distal promoter sequence of *Gallus gallus CCT6A* gene (GenBank accession number: NC_006106.3). For identifying polymorphisms in chicken *CCT6A* promoter region, two DNA pools were obtained by mixing 30 genomic DNA samples randomly with equal concentration from hens of Xinyang brown and Wenchang chicken population, respectively.

Polymerase chain reaction (PCR) amplification was performed in 20 μL volumes containing 2 μL of 10 \times Ex-buffer, 1.6 μL (2.5 mM) of dNTPs (TaKaRa, Dalian, China), 0.1 μL (5 U/ μL) of Ex-Taq DNA polymerase (TaKaRa, Dalian, China), 0.4 μL (10 μM) of each primer (CCT6A-P-F/R, Table 1), 1 μL genomic DNA pools (50–100 ng) and 14.5 μL of nuclease ddH₂O, and run on a Mastercycler gradient (Eppendorf, Germany) according to the following program: 94 $^{\circ}\text{C}$ for 4 min, 35 cycles of 94 $^{\circ}\text{C}$ for 30 s, annealing at 61 $^{\circ}\text{C}$ (CCT6A-P-F/R) for 30 s, and 72 $^{\circ}\text{C}$ for 45 s and final extension at 72 $^{\circ}\text{C}$ for 10 min. Fragments of 675 bp were resolved by electrophoresis with 1% agarose gel and purified with AxyPrepTM DNA Gel Extraction Kit (Axygen, Union City, CA, USA). The purified PCR product was inserted into pJET1.2/blunt-vector by Clone JETTM PCR Cloning Kit (Fermentas, Vilnius, Lithuania) according to the provided protocols. After being transformed into competent *E. coli* DH5 α cells, the recombinant plasmids were sequenced with BigDye[®] Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) by Jinan Bioson Co. Ltd. For each sample, at least 10 clones were sequenced. Sequences were aligned with the distal promoter sequence of *Gallus gallus CCT6A* gene (GenBank accession number: NC_006106.3) to identify nucleotide changes with DNAMAN v7.0.

Genotyping and Haplotype Analysis

Sequence alignment of chicken *CCT6A* promoter region revealed T>C mutations at sites -2215 and -1959 respectively (position designated according to translational initiation codon ATG). Using primer set CCT6A-P (Table 1), the polymorphisms caused by the two mutations were detected in Xinyang brown ($n=194$), Hy-line brown ($n=42$), Shouguang chicken ($n=49$), Wenchang chicken ($n=66$) and Luqin synthetic broiler line hens ($n=55$). The PCR pro-

Table 1. Primers used for molecular cloning, genotyping and expression analysis of chicken *CCT6A* gene

Primer name	Accession Numbers	Sequences (5'-3')	Size (bp)	Tm ($^{\circ}\text{C}$)	Usage
CCT6A-P-F	NC_006106.3	F: GCAGGTGTAGGATGGAAGAT	675	61	Molecular cloning and genotyping
CCT6A-P-R		R: CCACAAGTTTGGAGCCTTT			
qRT- β -actin-F	NM_205518.1	F: TGGATGATGATATTGCTGC	253	55	mRNA Expression
qRT- β -actin-R		R: ATCTTCTCCATATCATCCC			
qRT-CCT6A-F	NM_001006216.1	F: TTCCAGCCTTCCCAAACATA	128	55	mRNA Expression
qRT-CCT6A-R		R:GCCGTAGAATCACACTTTCAG			

cedure was described above. The PCR products were resolved on 1% agarose gel, purified with AxyPrep™ DNA Gel Extraction Kit (Axygen, Union City, CA, USA) and sequenced with BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) by Jinan Bioson Co. Ltd. Genotyping of each bird at the -2215 and -1959 sites in the distal promoter region of chicken *CCT6A* gene was carried out by checking the sequencing results. Haplotypes were constructed in the five chicken populations using the PHASE v2.0 program (Stephens *et al.*, 2001).

Tissues, RNA Isolation and cDNA Synthesis

To characterize the mRNA expression of chicken *CCT6A* gene of individuals with different genotypes, F1 (the biggest hierarchy follicle with mean diameter of about 33.75 mm), F5 (the smallest hierarchy follicle with mean diameter of about 12.60 mm) and POF1 (the newly post-ovulatory follicle) of 200-day-old Hy-line brown hens of each genotype were collected from chicken ovaries and stored in liquid nitrogen for total RNA extraction.

Total RNA was extracted from follicle tissues using TRIzol® Reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration of total RNA was measured with a spectrophotometer (Eppendorf, Hamburg, Germany). Its integrity was checked by using 1% agarose gel electrophoresis. The first strand cDNA was synthesized by PrimeScript™ RT Reagent Kit with gDNA Eraser (TaKaRa, Dalian, China) from 1 µg of total RNA mixed with 1 µL of oligo (dT)₁₈ primers, 1 µL of PrimerScript® RT Enzyme Mix I, 4 µL of 5 × Prime Script Buffer, 11 µL of RNase free ddH₂O and incubated at 37°C for 15 min and 85°C for 5 s. The resultant cDNA samples were stored at -20°C.

Real-time Quantitative PCR

The mRNA expression of *CCT6A* of individuals with different diplotypes was determined by real-time quantitative PCR (qRT-PCR), which was conducted on an Mx3000P (Stratagene, La Jolla, CA, USA) in a 15 µL volume containing 7.5 µL of 2 × SYBR® Premix Ex Taq™ (TaKaRa, Dalian, China), 0.3 µL of 50 × ROX Reference Dye II (TaKaRa, Dalian, China), 0.3 µL (10 µM) (for *CCT6A* and *β-actin*) of each forward or reverse primers (Table 1), 4.6 µL of ddH₂O, 2 µL of cDNA at a dilution of 1:9 according to the following program: 95°C for 30 s to activate the reaction and 95°C for 5 s, 55°C for 20 s and 72°C for 20 s for 40 cycles. The qRT-PCR efficiency was determined by the analysis of 3-fold serial dilutions of cDNA. Each qRT-PCR reaction was performed with a non-template negative control to check primer-dimers. The Ct in each reaction was determined by the baseline adjustment method of the MX3000 software (Stratagene, La Jolla, CA, USA). The 2^{-ΔΔCt} method was applied for calculating the relative gene expression levels under the condition that the efficiencies were nearly 100% (Livark and Schmittgen, 2001). The *β-actin* gene was used as the internal control with primers shown in Table 1. All samples were amplified in triplicate, and the obtained data were normalized to *β-actin* gene expression.

Statistical Analysis

The associations of *CCT6A* genotypes with egg production traits including AFE and E28 were analyzed in Xinyang brown chicken populations using the following General Linear Model of SAS (version 9.2; Cary, NC, USA), and the estimated genotype values were compared by Duncan's Multiple Range Test (SAS; version 9.2): $Y_{ij} = \mu + G_i + e_{ij}$, in which Y_{ij} is phenotypic value of traits, μ is the population mean, G_i is the fixed effect of genotype, e_{ij} is the random error effect. The difference between genotype effect were considered as significant when $P < 0.05$. The Hardy-Weinberg equilibrium (HWE) and heterozygosity were analyzed according to Nei's methods (Nei, 1973). Polymorphism information content (PIC) was calculated according to Botstein's methods (Botstein *et al.*, 1980). For qRT-PCR analysis, General Linear Model of SAS (version 9.2; Cary, NC, USA) was used to examine the difference of mRNA expression of chicken *CCT6A* gene among different haplotypes and when $P < 0.05$ the difference was considered as significant.

Results

Identification of Nucleotide Substitutions in the Distal Promoter Region of Chicken *CCT6A* Gene

A DNA fragment of 675 bp of the distal promoter region of chicken *CCT6A* gene was amplified from the DNA pools mixed from Xinyang brown and Wenchang hens, respectively. Sequence alignment of 10 clones from Xinyang brown chicken pool and 10 clones from Wenchang chicken pool indicates that two nucleotide substitutions exist between these two chicken breeds, i.e. g.-2215 T > C and g.-1959 T > C, respectively (position designated according to translational initiation codon ATG). Four haplotypes and six diplotypes were detected (GenBank accession numbers: KJ481888 to KJ481891) and shown in Fig. 1.

Alleles, Genotypes and Genetic Diversity

For the two polymorphisms located at g.-2215 T > C and g.-1959 T > C loci, totally 406 hens from five chicken breeds (lines) including Xinyang brown, Hy-line brown, Shouguang, Wenchang and Luqin synthetic broiler line were genotyped. For the polymorphism at g.-2215 T > C loci, allele T was predominant in Hy-line brown and Luqin synthetic broiler line, moderate in Xinyang brown, Wenchang and Shouguang hens, and deviation from HWE were observed in Xinyang brown, Hy-line brown and Luqin synthetic broiler lines ($P < 0.05$). For the polymorphism at -1959 site, allele C was moderate in Xinyang brown and Hy-line brown, relatively lower in Luqin synthetic broiler line and much lower in Shouguang and Wenchang chickens, and deviation from HWE was only observed in Xinyang brown line (Table 2).

Haplotype Analysis of Chicken *CCT6A* Gene

Four haplotypes including A (C⁻²²¹⁵-T⁻¹⁹⁵⁹), B (C⁻²²¹⁵-C⁻¹⁹⁵⁹), C (T⁻²²¹⁵-T⁻¹⁹⁵⁹) and D (T⁻²²¹⁵-C⁻¹⁹⁵⁹) were detected in all of the five chicken populations (Table 3), and the frequency of haplotype B was the lowest, only detected in Luqin synthetic broiler line. In Xinyang brown, haplotypes

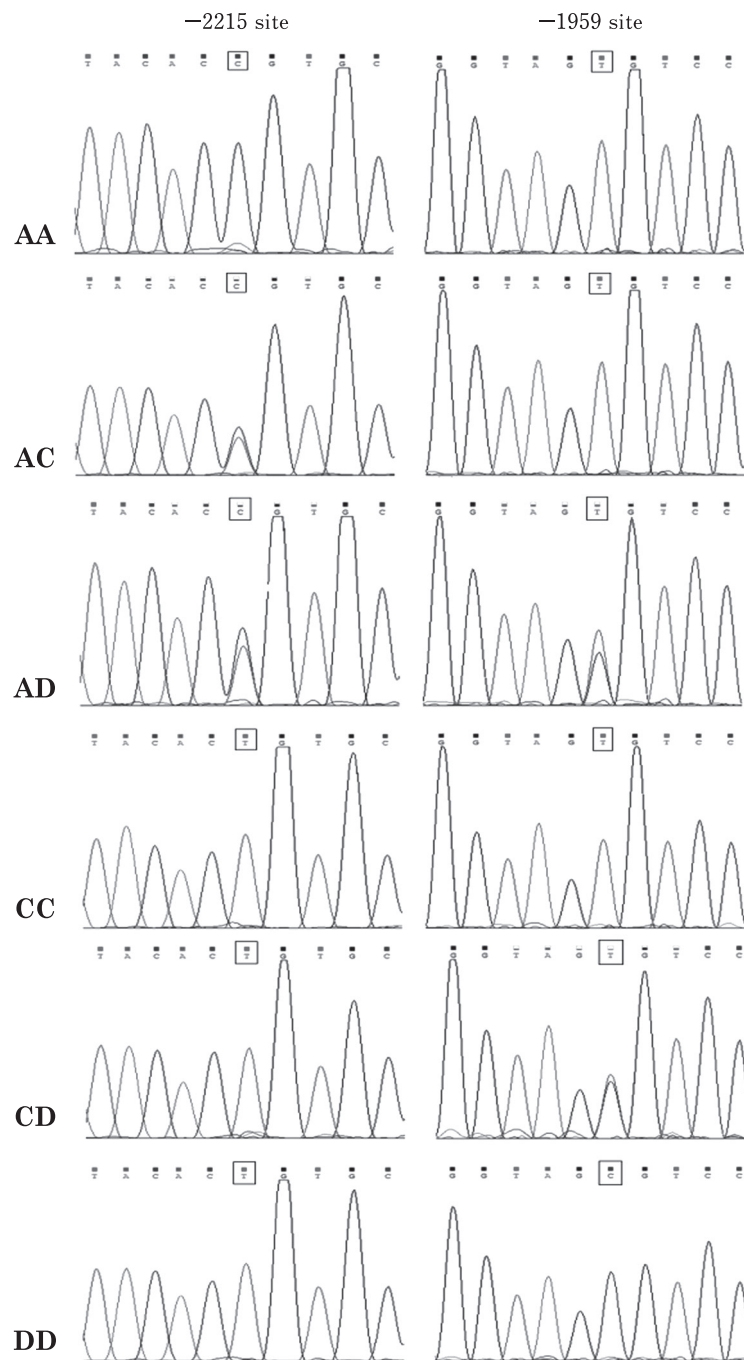


Fig. 1. Six diplotypes detected at -2215 and -1959 sites in the distal promoter region of chicken *CCT6A* gene in five hen populations. A = C⁻²²¹⁵-T⁻¹⁹⁵⁹; B = C⁻²²¹⁵-C⁻¹⁹⁵⁹; C = T⁻²²¹⁵-T⁻¹⁹⁵⁹; D = T⁻²²¹⁵-C⁻¹⁹⁵⁹.

A and D were more common than C. Haplotype D was predominant in Hy-line brown hens and haplotype C was predominant in Luqin synthetic broiler line hens. The distribution of haplotypes in Wenchang and Shouguang hens, which are two Chinese indigenous chicken breeds, was similar: haplotypes A and C account for $> 85\%$ in the two

populations.

Association of Diplotypes with Egg Production Traits

Associations between the polymorphisms at -2215 and -1959 sites of chicken *CCT6A* gene and egg production traits (AFE and E28) were analyzed in Xinyang brown chicken population (Table 4). The results indicated that individuals

Table 2. Genotype and allelic frequencies of chicken *CCT6A* gene's promoter region in five hen populations

SNPs	Population (number)	Genotype			Allele Frequencies		χ^2 (P value)	PIC	He
g.-2215 T>C		CC	CT	TT	T	C			
	Xinyang brown (194)	0.39	0.28	0.33	0.47	0.53	$\chi^2=34.68$ (P=9.999e-05)	0.3740	0.5000
	Hy-line brown (42)	0.06	0.20	0.74	0.84	0.16	$\chi^2=3.69$ (P=0.0209)	0.2357	0.2759
	Luqin (55)	0.11	0.22	0.67	0.78	0.22	$\chi^2=7.15$ (P=0.0164)	0.2830	0.3443
	Wenchang (66)	0.20	0.44	0.36	0.58	0.42	$\chi^2=0.61$ (P=0.4555)	0.3680	0.4898
	Shouguang (49)	0.24	0.62	0.14	0.45	0.55	$\chi^2=2.76$ (P=0.1417)	0.3724	0.5000
g.-1959 T>C		CC	CT	TT	C	T			
	Xinyang brown (194)	0.28	0.28	0.44	0.42	0.58	$\chi^2=30.75$ (P=9.999e-05)	0.3680	0.4875
	Hy-line brown (42)	0.31	0.52	0.17	0.57	0.43	$\chi^2=0.17$ (P=0.7751)	0.3707	0.4968
	Luqin (55)	0.09	0.38	0.53	0.28	0.72	$\chi^2=0.18$ (P=0.7577)	0.3229	0.4085
	Wenchang (66)	0.05	0.15	0.80	0.12	0.88	$\chi^2=5.50$ (P=0.0517)	0.1903	0.2147
	Shouguang (49)	0	0.16	0.84	0.08	0.92	$\chi^2=0.39$ (P=1.0000)	0.1387	0.1515

Table 3. The distribution of haplotypes in five different hen populations

Population (number)	A (C-T)	B (C-C)	C (T-T)	D (T-C)
Xinyang brown (194)	0.5274	0	0.0576	0.4150
Hy-line brown (42)	0.1631	0	0.2717	0.5652
Luqin (55)	0.2091	0.0091	0.5091	0.2727
Wenchang (66)	0.4091	0	0.4545	0.1364
Shouguang (49)	0.5511	0	0.3673	0.0816

Table 4. Least squares means for age at first egg (AFE) and egg number at 28 weeks of age (E28) among diplotypes in the promoter region of the *CCT6A* gene in Xinyang brown hens

Diploypes	No. of birds	AFE	E28
AA (C-T/C-T)	72	161.7±0.9	25.3±0.9 ^b
AD (C-T/T-C)	50	158.4±1.7	29.8±1.1 ^a
DD (T-C/T-C)	58	161.7±1.4	26.8±1.3 ^{ab}
P value		0.0863	0.0182

Data with different superscript letters are significantly different ($P<0.05$).

with diplotype AD laid more eggs at 28 weeks (E28) than the ones with diplotype AA and DD ($P<0.05$), however its effect on AFE was not significant ($P>0.05$) (Table 4).

Comparison of mRNA Expression of *CCT6A* Gene in Follicles from Hens with Different Haplotypes

The mRNA expression of chicken *CCT6A* gene in F1, F5 and POF1 of 200-day-old Hy-line brown hens was analyzed and compared among hens carrying diplotypes AA, AD and

DD. The results indicated that, *CCT6A* mRNA level was the highest in birds carrying diplotype AD than that in the ones carrying diplotypes AA and DD, respectively ($P<0.05$) (Fig. 2).

Discussion

The reported function of *CCT6A*, as a hetero-oligomeric molecular chaperone, is to assist the folding of cytoplasmic

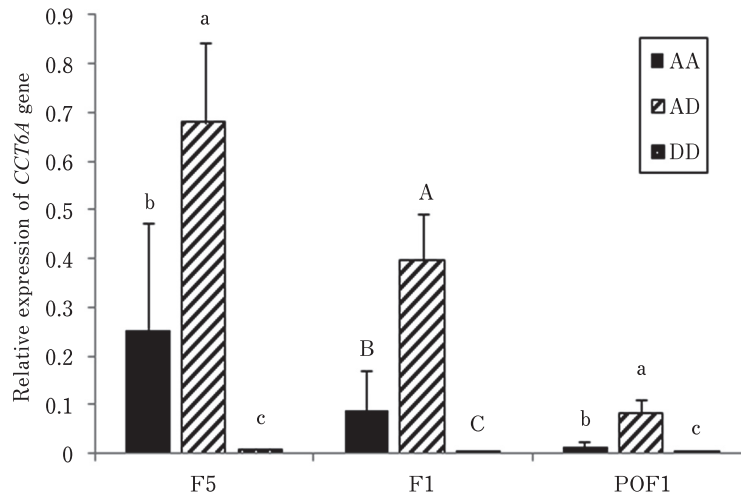


Fig. 2. Comparison of mRNA expression of chicken *CCT6A* gene in F1 (the biggest hierarchy follicle with mean diameter of about 33.75 mm), F5 (the smallest hierarchy follicle with mean diameter of about 12.60 mm) and POF1 (the newly post-ovulatory follicle) of 200-day-old Hy-line hens with diplotypes AA ($n=3$), AD ($n=4$) and DD ($n=4$). Means \pm SD were calculated for each diplotype. Bars with different superscript letters (a, b or A, B) mean significantly different ($P < 0.05$ or $P < 0.01$).

proteins. Studies showed that its expression is related to tumor in human cancer cell lines (Myung *et al.*, 2004; Zhang and Lin, 2006) and reproduction in mice (Dun *et al.*, 2011). Its role in bird reproduction was primarily recognized by two studies from our group finding that its expression is consistent with follicle growth and ovulation (Kang *et al.*, 2012; Wei *et al.*, 2013). We also found that the expression of chicken *CCT6A* is regulated by progesterone and follicle-stimulating hormone, and a progesterone response element was located in its distal promoter region (Wei *et al.*, 2013). In this study, the polymorphisms in chicken *CCT6A* promoter region and their genetic effects on egg production and mRNA expression were further analyzed.

Two novel SNPs in the distal promoter of chicken *CCT6A* gene (g.-2215 T>C and g.-1959 T>C) were observed in five chicken breeds (lines). Totally four haplotypes and six diplotypes were detected in these populations. The distribution of haplotypes A, B, C and D were remarkably different in high-egg-production chicken breed (Hy-line brown and Xinyang brown), broilers (Luqin synthetic broiler line) and two Chinese indigenous chicken breeds (Wenchang and Shouguang). To analyze associations of diplotypes with egg production traits, we obtained a population by crossing heterozygous Xinyang brown chickens at these two polymorphic sites. The results showed that, compared with diplotypes AA and DD, diplotype AD was associated with higher E28 and lower AFE, suggesting that dominant effect exists at these two sites of chicken *CCT6A* gene. Complex quantitative traits of animals are affected not only by single SNP, but also by SNP-SNP interactions of candidate genes

(Huang *et al.*, 2011). Because the two SNPs identified in this study are located at -2215 and -1959 of chicken *CCT6A* gene, they might affect transcription level *via* altering the transcription factor binding capacity, and, the higher expression of *CCT6A* mRNA in diplotype AD hens is likely caused by the interactions between transcription factors binding to these two sites. Consistently, diplotype AD was also predominant in commercial laying hens with high egg production, *i.e.* Xinyang brown and Hy-line brown chicken populations.

We further compared the mRNA expression of chicken *CCT6A* gene in follicles among Hy-line brown hens of different diplotypes as neither ovary nor follicle tissues of Xinyang brown hens were available. In the F1, F5, and POF1 follicles, significant differences in *CCT6A* mRNA expression were detected among diplotypes AA, AD and DD, the mRNA expression of *CCT6A* being the highest in diplotype AD birds and the lowest in diplotype DD birds. The mRNA expression of *CCT6A* in the F1, F5, and POF1 follicles is consistent with its effect on egg production at 28 weeks (E28). In our previous study, the highest mRNA level of chicken *CCT6A* gene was also found in Hy-line brown ovary during peak egg laying stage (Kang *et al.*, 2012). In another study, we found a significant higher expression of *CCT6A* mRNA in theca cells than in granulosa cells of chicken ovarian follicle, and in theca cells, knock-down of *CCT6A* decreased Bcl-2 expression (Yang *et al.*, 2015), suggesting that *CCT6A* likely affect egg production by stimulating the proliferation of theca cells. Wei *et al.* (2013) found that progesterone can activate *CCT6A* transcription by

the progesterone response element (PRE) located from -2056 to -2051 of chicken *CCT6A* gene promoter. The two mutations at -2215 and -1959 sites reported by this study were located at the 5' - and 3' -flanking regions of PRE of the distal promoter of chicken *CCT6A* gene, respectively; therefore, may interfere the binding of PGR or other transcriptional factors to the promoter, or interact with other polymorphisms regulating *CCT6A* gene expression, which requires further studies.

In conclusion, haplotypes caused by two single nucleotide polymorphisms (SNPs) at the -2215 and -1959 sites of chicken *CCT6A* gene promoter were revealed. The frequencies of haplotypes and diplotypes were different between chicken breeds (lines) differing in egg production. Individuals with diplotype AD laid more eggs at 28 weeks than the ones with diplotypes AA and the mRNA expression of *CCT6A* in diplotype AD birds is the highest in F1, F5, and POF1 follicles of Hy-line brown hens. These results suggest that the two SNPs in the distal promoter region of chicken *CCT6A* gene could be used as DNA markers in chicken breeding to improve egg production trait.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 31272435), Agricultural Elite Breeds Project of Shandong Province (2015) and the Innovation and Application Research of Poultry Grant of Shandong province. We appreciate the help from Huaxiang Yan and Changsuo Yang for collecting Xinyang brown chicken samples.

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