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THE ASSOCIATION BETWEEN NOVEL POLYMORPHISMS OF GREMLIN GENES AND EGG-LAYING PERFORMANCE TRAITS IN CHINESE VILLAGE DAGU HENS

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Abstract

Gremlin (*GREM1*, *GREM2*) genes are the known bone morphogenetic proteins (BMPs) inhibitors, but their genetic diversity in animal species remains unknown. The current study was conducted to investigate single nucleotide polymorphisms (SNPs) in chicken *GREM1* and *GREM2* genes, and their association with egg production traits using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and deoxyribonucleic acid (DNA) sequencing. The results discovered novel SNPs and, among these variations, C/T transition at position 436 in exon 1 of the *GREM1* gene leads to synonymous substitution of amino acids, and T/C transition at position 690 in the coding region of the *GREM2* gene leads to a non-synonymous substitution of amino acids (valine acid 114-to-alanine acid). Association analysis established that at the age of 43, 57 and 66 wks, hen-house egg production (HHEP) was more highly significantly associated (P<0.05) with the AA genotype in the *GREM1* gene. In the *GREM2* gene, the TC genotype was remarkably linked with higher HHEP at the age of 30, 57 and 66 wks. Our results provide evidence that the *GREM1* and *GREM2* genes have potential effects on HHEP in chickens. SNPs determined in this work may be utilised as favourable potential DNA markers for improving of egg-laying performance traits.

Key words: Chinese Dagu chicken, egg-laying performance, *GREM1*, *GREM2*, polymorphisms

Egg production is a product of many genes performing on large number of biochemical processes (Fairful and Gowe, 1990), and is one of the most vital profitable traits in chicken layers (Kim et al., 2004; Qin et al., 2015; Jing et al., 2016). Over the last decades, genetic improvement of egg production traits in chickens has been made by selection based on phenotype or estimated breeding values. However, the recent developments of molecular genetics have established molecular markers for improving egg production traits in chicken breeding. Nevertheless, egg production traits – due to their low to moderate heritability – are very difficult to improve geneti-

cally (Venturini et al., 2013), Many researchers have indicated that a candidate gene approach for investigating the correlation between sequence variations and quantitative traits loci (QTLs) accountable for the difference in traits of interest is cost-effective (Linville et al., 2001; Rothschild and Soller, 1997). Hence, the problem has drawn more attention from poultry breeders, focusing on egg production all over the world, to explore the candidate genes associated with egg production traits by identifying the single nucleotide polymorphisms (SNPs) (Kim et al*.,* 2004; Zhou et al., 2008; Xu et al., 2010; Mu et al., 2015; Qin et al*.,* 2015; Jing et al., 2016; Niu et al., 2017). In chicken breeding for marker-assisted selection (MAS), several SNPs and candidate genes are necessary, and some progress has been made in animal breeding by using the MAS method.

Gremlin (*GREM1*, *GREM2*) genes are DAN family members which are the antagonist of bone morphogenetic proteins (BMPs) (Segditsas et al., 2008; Myers et al., 2011; Kattamuri et al., 2012; Nilsson et al., 2014). The *GREM1* and *GREM2* genes have been reported for their significant role in mice, rats and humans (McMahon et al., 1998; Sudo et al., 2004; Cook et al., 2007; Gazzerro and Minetti, 2007; Mine et al., 2008; Yuasa and Fukuda, 2008; Nilsson et al., 2014). In addition, Huillard and Marx (2004) reported the importance of the chicken *GREM1* and *GREM2* genes during the chicken embryo development. In chicken, the *GREM1* gene is situated on chromosome 5 which encodes a Gremlin-1 protein; this gene has only one exon encoding with 184 amino acid polypeptides, while the *GREM2* gene is located on chromosome 5 which encodes a Gremlin-2 protein with 2 exons 179 amino acid polypeptides (International Chicken Genome Sequencing Consortium, 2004). However, based on our knowledge no previous study has been done in identifying SNPs on the *GREM1* and *GREM2* genes linked with egg production characteristics in hens.

The Chinese Dagu chicken breed is one the most common indigenous Chinese chicken breeds (Wu et al., 2004; Tyasi et al., 2017; Niu et al., 2017) that is widely distributed in the north-east area of China (Qu et al., 2004). This breed is well-known for its adaptation superiority in terms of cold weather and is a good egg producer (Li et al., 2009; Tyasi et al., 2017). This indicates that this local chicken is an extremely significant farm animal that is reserved for a source of animal protein and income for most rural communities. Previous studies have been performed to investigate the SNPs and their association with egg production traits in the Chinese indigenous Dagu chicken population (Qin et al., 2015; Mu et al., 2015; Jing et al., 2016; Niu et al., 2017). However, genetic polymorphisms characterisations within the *GREM1* and *GREM2* encoding regions in this chicken breed have been poorly investigated. According to Honarvar et al. (2012), detection of SNPs is one of the most important investigations for genetic improvement because nucleotide substitutions in the genome may perhaps alter the level of gene expression.

The objectives of the current study were: 1) to identify the SNPs in the chicken *GREM1* and *GREM2* genes using polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) and deoxyribonucleic acid (DNA) sequencing, and 2) to investigate the association of SNPs with egg production traits in the Chinese Dagu chicken population. This study will provide a basic literature on genetic

diversity of gremlin genes for further studies in other chicken breeds and also any animal species.

Material and methods

Birds and measurements of traits

The College of Animal Science and Technology at Jilin Agricultural University provided the chicken breed for the study. Eggs from the Chinese Dagu chickens were randomly selected and hatched, and about 360 chicks were randomly selected after hatching. All the chickens for the study were raised in layered battery cages with free access to water, and the feeding process was carried out according to the nutrient requirements of local Chinese chickens. The hens were reared separately in maintained conditions when approaching 16 weeks of age. At 5:00, almost every day all the chickens were unsheltered for lights on to a 16L: 8D photoperiod. As soon as the chickens had finished laying eggs, all the eggs per pen were collected daily and registered. At the age of 30 and 43 weeks laying performance was computed for each bird, and after the feed and water restrictions body weight was estimated. In all the hens, at 30, 43, 57 and 66 weeks of age, egg production traits were tested such as egg weight, egg laying number (hen-housed egg production), and at 30 and 43 weeks of age individual body weight was recorded. Table 1 shows the summary of the egg production traits and body weight recorded in this study. Mortality was also recorded during the study. All protocols performed on the chickens were approved by the Institutional Animal Care and Use Committee of Jilin Agricultural University, Changchun, China (IUCAC Number: JLAU20140921).

Table 1. Descriptive statistics of egg production traits of the Chinese indigenous Dagu hens

Egg production traits	Mean±SD	CV	Minimum	Maximum
BW at 30 wks (kg)	2.79±0.32	11.45	2.20	3.88
BW at 43 wks (kg)	2.97±0.54	18.14	1.54	4.78
HHEP at 30 wks (no.)	21.40±13.69	63.95	2.00	55.00
HHEP at 43 wks (no.)	85.54±36.07	42.16	6.00	148.00
HHEP at 57 wks (no.)	125.39±52.63	41.98	6.00	205.00
HHEP at 66 wks (no.)	136.51±60.04	43.98	7.00	241.00
EW at 30 wks (g)	56.23±4.38	7.79	47.80	64.90
EW at 43 wks (g)	59.69±4.15	6.95	52.90	72.80

BW = body weight; HHEP = hen-housed egg production (total egg laying number); EW = egg weight; CV = coefficient of variation; SD = standard deviation; wks = weeks.

Samples of DNA and amplification of PCR

360 Chinese Dagu hens were sampled for the current study. At the age of 300 d, about 1 ml of blood from a wing vein was collected and, following the standard phenol-chloroform method, the DNA was separated from the blood samples. Ultra-

violet-spectrophotometer assay and 1% agarose gel electrophoresis were conducted to find genomic DNA purity, and the detection achieved concentrations which were between 2 and 10 ng/µl. All the primers for the current work were designed based on the candidate genes (*GREM1*: GenBank accession No. NC_006092.4 and *GREM2*: GenBank accession No. NC_006090.4) sequences in chickens. Table 2 shows the primer pairs which were utilised to intensify the fragment for the *GREM1* and *GREM2* genes.

Table 2. Primer information of the fragment of chicken *GREM1* and *GREM2* genes amplified

Genes	Sequences of primers (5'-3')	Product length (Position)	Annealing temperature (°C)
GREM1	F: CCCTCAAACAAACTATCCA	250 bp (289–532 nt)	54
	R: ATACACCGACACTCCTTCA		
GREM2	F:CGCACCATCATCAACCGCTTCT	191 bp (441–631 nt)	63
	R: TGCACCGGCACTGCTTCACC		

A volume of 50 µl, including: 100 nM of each primer, 22 µl RNase-free Water, 20 to 50 ng of template DNA, 20 µl of 2× Taq Master Mix and the PCR reactions were executed. For the PCR reactions, the conditions were as follows: 94°C for 2 min, 35 cycles at 94°C for 30 s for denaturing, 63°C (54°C) for 30 s for annealing, 72°C for 30 s for extension, and then the last extension at 72°C for 2 min.

PCR products cloning, sequencing followed by alignment

The Wizard® prep PCR purification system (Promega, Madison, WI, USA) was exploited to purify the PCR products, according to the method of Sambrook and Russell (2001), the Promega pGEM®-T easy vector was used for cloning, then after cloning the fragments were sequenced commercially. BLAST (http://blast.ncbi.nlm.nih.gov) was used to confirm the predictable chicken *GREM1* and *GREM2* gene sequences, and two PCR independent amplifications were performed for each of the sampled chickens. DNAMAN software version 6.0 was used for alignment of the sequences to recognise nucleotide changes.

Genotyping and combined genotypes reconstruction

Single-strand conformation polymorphism (SSCP) assay was carried out for analysis on confirmed PCR products for screening gene polymorphism. For this, 6 µl of loading dye (98% deionized formamide, 0.025% bromophenol blue, 2% glycerin, 10 mM EDTA and 0.025% xylene cyanol) was mixed with 10 µl PCR products. The solutions were denatured at 98°C for 16 min, then cooled quickly on ice and loaded on 12% polymerised gels (acrylamide: bisacrylamide, 39:1) 16 × 18 cm of size 16. Electrophoresis in about 1× TBE buffer was conducted at 120 V for 5 h at room temperature. Gene Snap from SynGene (Gel photography system) was exploited to detect the gels after silver staining. In the current study, each DNA sample was often amplified and detected to avoid false results. According to Stephens et al. (2001), PHASE Program was conducted for reconstruction of combined genotypes accordance to the genotyped data of all the 360 hens used in the present study.

Assessment of polymorphism

Allelic and genotype frequencies were measured at each SNP site, then Pearson's goodness-of-fit chi-square test (degree of freedom = 1) was performed to test each SNP for Hardy-Weinberg equilibrium. According to Yeh et al. (1997), POPGENE v.1.32 software was utilised for statistical analyses of polymorphism information content (*PIC*), effective number of alleles (*Ne*), gene homozygosity (*Ho*) and heterozygosity (*He)*.

Analysis of marker-trait association

General linear model (GLM) method in SPSS 18.0 was exploited for association analyses of sequence variations or combined genotypes with egg production traits. The model used was as follows:

$$Y_{ij} = \mu + A_i + e_{ij}$$

where:

Y_{ij} is the trait phenotypic value, such as body weight,

μ is the mean population,

A_i is the fixed effect of the ith genotype or combined genotype,

e_{ij} is the residual error.

In each test the sum of square of Type III was utilised. Statistical significance was computed at $P<0.05$ for the current study.

SNP genotype effects prediction

According to Falconer et al. (1996) mean differences between allelic and genotype frequencies were conducted to forecast additive effects for the polymorphisms that showed statistical significant relationship with egg production traits. The following formula was utilised to find additive genetic variance percentage ($\%V_j$) elucidated by single nucleotide polymorphisms.

$$\%V_j = 100p_jq_j\alpha_j\text{^}2/V_g$$

where:

q and p are the allelic frequencies for the jth SNP predicted,

α_j – predicted genetic additive effect of the jth SNP,

V_g – restricted maximum likelihood (REML) estimate of the (poly-) genetic variance for trait.

Descriptive statistics

Means, standard deviation (SD), minimum, maximum and coefficient of variation (CV) of the egg production traits were computed as descriptive statistics (Table 1) using SPSS Version 18.0.

Results

Nucleotide sequence amplified analysis

In the current study, specifically designed primers of about 250 bp for *GREM1* and 191 bp fragments for *GREM2* were cloned. BLAST software was performed to compare the predicted sequences with GenBank sequences (NC_006092.4 for *GREM1* and NC_006090.4 for *GREM2* gene). Figure 1 and Table 2 demonstrate that the primers used for the present study were confirmed with the PCR amplicon sizes related to the predictable gene sequences. PCR product sequences were analogised with direct genomic PCR product for further confirmation. Nucleotide sequence amplified results proved that only two sequences of alleles were established for all the investigated hens, and signified that the primer pair used for each candidate gene was largely amplified for a particular gene.

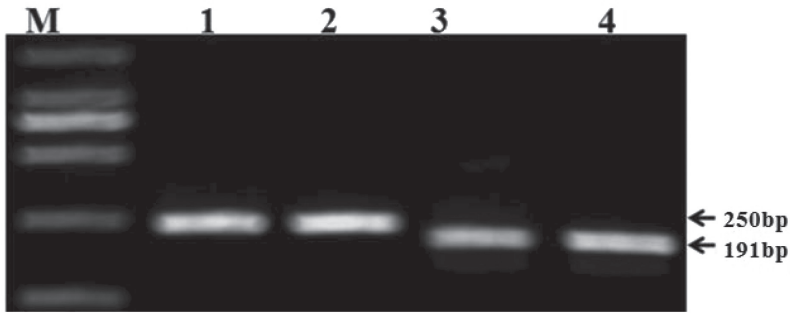

Figure 1. Chicken *GREM1* and *GREM2* fragments amplification. M, DL 2000 DNA marker (2,000; 1,000; 750; 500; 250 and 100 bp, respectively.); Lanes 1–2, fragments of *GREM1* gene amplified; Lanes 3–4, fragments of *GREM2* gene amplified

Genotyping and combined genotypes reconstruction

PCR-single strand confirmation polymorphism analysis was conducted for genotyping of the amplified *GREM1* and *GREM2* fragments. In exon 1 of *GREM1* the results showed AA genotype and AB genotype (Figure 2 a), and in the coding region of *GREM2* established TT genotype and TC genotype (Figure 3 a). Genotype data was utilised for the reconstruction of combined genotypes and the results confirmed about 4 combined genotypes (AATT, ABTT, AATC and ABTC), which were recognised among all the 360 birds. AATT (0.56) was recognised as the highest frequency combined genotype, followed by ABTT and ABTC and the lowest frequency combined genotype negated the AATC, respectively.

Sequence variation analysis

For polymorphisms of the target sequences, PCR-SSCP banding patterns were performed followed by DNA sequencing. The results demonstrated a C/T transition on the *GREM1* fragment at the coding region of the exon 1 base 436 site (Figure 2 b) and referred to as SNP C436T. This noted that C/T transition implicate to the syn-

onymous amino acid exchange. In all the 360 hens, PCR-SSCP analysis confirmed that the SNP in *GREM1* recognised only two genotypes (AA and AB). These results also proved a T/C transition on the *GREM2* fragment at the coding region base 690 site (Figure 3 b) and referred as SNP T690C. PCR-SSCP analysis results revealed two chicken genotypes for *GREM2* (TT and TC). The results also established that SNP T690C on *GREM2* leads to a non-synonymous amino acids exchange (valine acid 114-to-alanine acid).

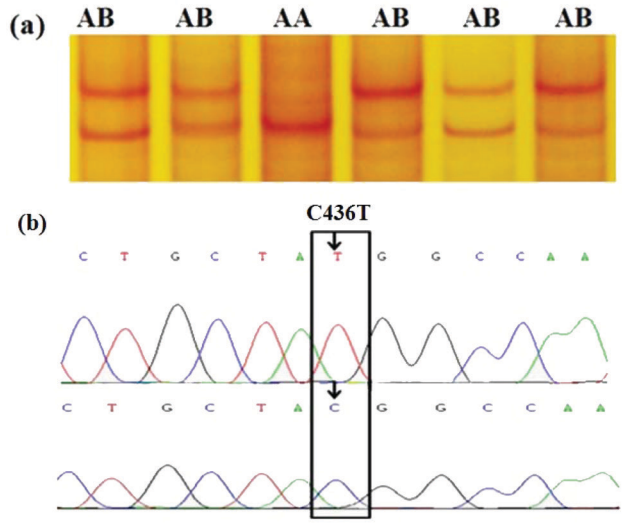

Figure 2. PCR-SSCP band patterns and the SNP C436T site in the chicken *GREM1* gene. (a) PCR-SSCP band patterns; (b) the C436T transition in exon 1 of the chicken *GREM1* genes

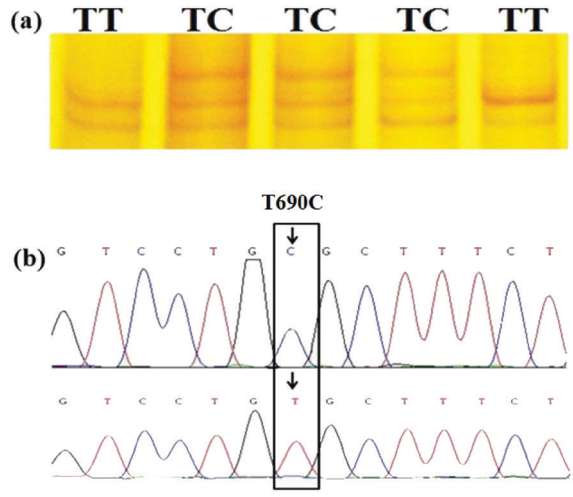

Figure 3. PCR-SSCP band patterns and the SNP T690C site in the chicken *GREM2* gene. (a) PCR-SSCP band patterns; (b) the T690C transition in the coding region of the chicken *GREM2* gene

Genotypic and allelic frequencies

Table 3 confirmed the allelic and genotypic frequencies for the candidate genes. The results proved that a frequency of the A allele revealed a higher frequency than the B allele at *GREM1*, with the AA genotype establishing a higher genotypic frequency than the AB genotype. While allele T confirmed a higher frequency than allele C, TT genotype showed higher frequency than TC genotype at *GREM2*. SNPs were tested and demonstrated remarkable genetic disequilibrium between alleles ($P<0.05$), respectively.

Table 3. Genotypic and allelic frequency at the SNP locus of the *GREM1* and *GREM2* genes in the Chinese local Dagu chicken population

SNP	Genotype	No. of chickens	Genotype frequency	Allele	Allele frequency	X^2
C436T *(GREM1)*	AA	280	0.778	A	0.889	5.63*
	AB	80	0.222	B	0.111	
T690C *(GREM2)*	TT	242	0.672	T	0.836	13.83*
	TC	118	0.328	C	0.164	

*$P<0.05$ was accepted to be statistically significant when the data were analysed using a Pearson's goodness-of-fit chi-square test (degree of freedom = 1).

Table 4. Polymorphism information analysis of chicken *GREM1* and *GREM2* genes in the Chinese local Dagu chicken population

SNP	Gene homozygosity (*Ho*)	Gene heterozygosity (*He*)	Effective allele number (*Ne*)	Polymorphism information content (*PIC*)
C436T *(GREM1)*	0.802	0.222	1.246	0.178
T690C *(GREM2)*	0.726	0.328	1.378	0.237

Table 4 reveals that gene heterozygosity (*He*) was lower than gene homozygosity (*Ho*) for both candidate genes, with the 1.246 (P1) and 1.378 (P2) effective allele number. The results also proved that the polymorphism information content (PIC) value of *He* in P2 (*GREM2*) was higher than the value of P1 (*GREM1*).

SNP genotypes association with laying performance and SNP genotypic effects prediction

Association analysis demonstrated that between egg production traits and single nucleotide polymorphisms (Table 5) on *GREM1*, at 43, 57 and 66 wks of age, AA genotype was remarkably related with higher hen-housed egg production (HHEP), and at 43 wks of age confirmed association with lower body weight (BW) ($P<0.05$), respectively. In addition, at 30, 57 and 66 wks of age TC genotype at *GREM2* revealed a statistically significant association with the higher HHEP ($P<0.05$), respectively. SNP justified the greater additive genetic variance percentage (Table 6) and discovered a noteworthy relationship with the egg-laying performance traits (>1%).

In Table 6, a distinction was only found in BW at 43 wks of age and in HHEP at 43, 57 and 66 wks of age between the AA and AB genotypes. The results showed that a significant difference was also recognised in HHEP at 30, 57 and 66 wks of age between TT and TC genotypes (P<0.05), respectively.

Table 5. Association between the polymorphism in chicken *GREM1* and *GREM2* genes and egg production traits in the Chinese local Dagu hens

Egg production traits	Genotypes			
	GREM1		*GREM2*	
	AA (280)	AB (80)	TT (242)	TC (118)
	($\bar{x}$± SE)	($\bar{x}$± SE)	($\bar{x}$± SE)	($\bar{x}$± SE)
BW at 30 wks (kg)	2.77±0.05	2.84±0.07	2.75±0.05	2.85±0.07
BW at 43 wks (kg)	2.85±0.08 b	3.23±0.14 a	2.97±0.09	3.03±0.12
HHEP at 30 wks (no.)	21.69±2.05	20.44±3.83	19.06±2.28 b	31.88±4.16 a
HHEP at 43 wks (no.)	86.05±6.20 a	62.22±7.52 b	82.90±7.44	85.88±6.53
HHEP at 57 wks (no.)	126.92±8.94 a	77.61±9.91 b	97.81±10.05 b	131.60±10.16 a
HHEP at 66 wks (no.)	137.90±10.21 a	89.06±9.24 b	97.84±9.50 b	143.64±11.61 a
EW at 30 wks (g)	56.57±0.52	56.39±1.09	56.58±0.83	56.35±0.85
EW at 43 wks (g)	59.57±0.52	59.96±1.36	59.49±0.69	59.93±0.93

a, b – means within a row for each gene lacking a common letter differ (P<0.05). The no. represents the total number of eggs at the corresponding age. Numbers in the parenthesis indicate the number of hen individuals in each group: n = Sample size, BW = body weight, HHEP = hen-housed egg production (total egg laying number), EW = egg weight. The 30 wks of age is the average age at first egg of the Dagu hens.

Table 6. Percentages of additive genetic variance explained by the SNPs identified in the fragments of chicken *GREM11* and *GREM2* genes

Egg production trait	Percentage of additive genetic variance explained by the SNPs (%)	
	C436T (*GREM1*)	T690C (*GREM2*)
BW at 30 wks (kg)	–	–
BW at 43 wks (kg)	2.526	–
HHEP at 30 wks (No.)	–	5.633
HHEP at 43 wks (No.)	3.601	–
HHEP at 57 wks (No.)	5.799	3.691
HHEP at 66 wks (No.)	5.551	6.782
EW at 30 wks (g)	–	–
EW at 43 wks (g)	–	–

Among the four combined genotypes (Table 7), at the age of 30, 43, 57 and 66 wks the highest HHEP proved a remarkable association with combined genotype 3 (H3) and 4 (H4) (P<0.05), respectively. The results at 43 wks of age in BW demonstrated no variation between combined genotype H1 and H3, and between H2 and H4 (P>0.05), respectively. Combined genotypes results also confirmed no noteworthy

relationship between all the four combined genotypes at 30 wks of age in BW, at 30 and 43 wks of age in egg weight (P>0.05), respectively.

Table 7. Association between the combined genotypes with egg production traits in the Dagu hens

Egg production trait	Combined genotypes			
	H1 (202)	H2 (18)	H3 (95)	H4 (45)
	($\bar{x}$± SE)	($\bar{x}$± SE)	($\bar{x}$± SE)	($\bar{x}$± SE)
BW at 30 wks (kg)	2.76±0.05	2.76±0.09	2.79±0.09	2.89±0.14
BW at 43 wks (kg)	2.86±0.09 b	3.23±0.17 a	2.82±0.14 b	3.23±0.21 a
HHEP at 30 wks (no.)	21.35±2.64 ab	12.57±3.38 b	22.38±3.29 ab	25.46±5.47 a
HHEP at 43 wks (no.)	85.81±8.30 a	77.00±8.29 b	86.54±8.82 a	89.18±8.69 a
HHEP at 57 wks (no.)	107.14±11.37 b	122.58±5.89 ab	131.55±5.55 a	135.62±10.51 a
HHEP at 66 wks (no.)	116.71±10.07 b	132.89±13.12 ab	144.18±10.28 a	147.92±11.05 a
EW at 30 wks (g)	56.37±0.89	57.47±1.69	55.73±1.14	55.70±1.40
EW at 43 wks (g)	59.29±0.60	60.40±2.18	60.13±1.00	59.68±1.82

a, b – means within a row for each gene lacking a common letter differ (P<0.05). The no. represents the total number of eggs at the corresponding age. Numbers in brackets indicate the number of hen individuals in the group. H1 = AATT; H2 = AATC; H3 = ABTT; H4 = ABTC.

Discussion

The current study investigates for the first time the SNPs of chicken *GREM1* and *GREM2* genes in the Chinese Dagu chicken breed population using PCR-SSCP and DNA sequencing. Our results found one SNP (C436T) in exon 1 with AA and AB genotypes in the *GREM1* gene, while also establishing one SNP (T690C) in the coding region with TT and TC genotypes in the *GREM2* gene. SNP C436T (*GREM1*) results in synonymous replacement of amino acid, and SNP T690C (*GREM2*) implicates a non-synonymous substitution of amino acids (valine acid 114-to-alanine acid). These findings suggest that changes in amino acids sequences might affect the protein structure and function. Chi-square test results demonstrated that the allelic and genotypic frequencies for both genes were not in Hardy-Weinberg equilibrium. These results suggest that the allelic and genotypic frequencies of the *GREM1* and *GREM2* genes of the Chinese Dagu chicken population do not remain constant from generation to generation due to the influence of selection, mate choice, migration and mutation. SNPs were tested and demonstrated remarkable genetic disequilibrium between alleles which might change the population structure and genetic drift in a small population.

In the 360 birds, the AA genotype had higher frequency than the AB genotype and the A allele was higher than the B allele in the *GREM1* gene. In addition, the TT genotype had a higher frequency than the TC genotype and the T allele was higher than the C allele in the *GREM2* gene.

A limitation of the current study was the unavailability of any literature on the genetic diversity of the *GREM1* and *GREM2* genes in any animal species. There-

fore, our data will provide for the first time the relevant literature for further studies. However, our previous study on the Chinese Dagu chicken population investigated SNPs on estrogen receptor 1 and 2 genes, and identified SNP (T1101C) within exon 4 of estrogen receptor 1 gene and SNP (G1755A) within exon 8 of estrogen receptor 2 (Niu et al., 2017). Furthermore, Qin et al. (2015) investigated two candidate genes in Chinese Dagu hens and discovered two novel SNPs, one at the base position 238 of the chicken *FOXL2* gene and the other one at the position 1609 of the chicken *GDF9* gene.

The association analysis results of the current study revealed that in the *GREM1* gene, the AB genotype had a significant effect on body weight at a mature age, while the AA genotype had a significant influence on the total number of eggs. Although the genotypes in the *GREM2* gene had no significant effect on the body weight of the Chinese Dagu indigenous hens, the heterozygous (TC) genotypes had a more significant effect on the total number of eggs than the TT genotypes. Our data revealed that both genes had no significant effect on egg weight. These results suggest that the AA genotype in the *GREM1* gene may be used as a potential genotype for improving body weight, while the AB genotype might be used for improving the total number of eggs. However, the TT genotype in the *GREM2* gene might not be used for improving body weight, but the TC genotype might be used for improving the total number of eggs.

A previous study which investigated the association between SNPs and egg production traits on the Chinese Dagu indigenous chicken population demonstrated that the periostin (*POSTN*) and platelet-derived growth factor receptor-like (*PDGFRL*) genes had no significant effects on body weight, while the AA genotype had a more significant effect on the total number of eggs than the AB genotype in the *POSTN* and *PDGFRL* genes, the TT genotype had a more significant effect on the total number of eggs than the TC and CC genotypes, although the CC genotype had a significant influence on body weight and egg weight (Jing et al., 2016). Our own previous study demonstrated the relationship between the SNPs of the estrogen receptor 1 (*ESR1*) and 2 (*ESR2*) genes with egg production traits, and suggested no significant effects on body weight in both genes. Additionally, the TT genotype had a more significant effect on the total number of eggs than the CT genotype in the *ESR1* gene, while the GG genotype in the *ESR2* gene had a more significant effect on total number of eggs than the GA genotype (Niu et al., 2017).

The current study also identified four combined genotypes and their correlation analysis which confirmed that egg production traits were statistically significantly associated with *GREM1* and *GREM2* polymorphisms. These combined genotypes outcome might be used as possible DNA markers for improvements in egg production in chicken breeding.

We exploited the SNPs to estimate the additive genetic variance percentage in the Chinese Dagu chickens and the SNPs clarified a noteworthy correlation of egg production traits with the greater additive genetic variance percentage (>2.5%).

Conclusions

The current study found two novel SNPs of gremlin genes in 360 Chinese Dagu hens by using PCR-SSCP analysis and genomic DNA sequencing, and identified that

the SNPs of gremlin genes influence egg production traits. This study not only provides the candidate genetic markers for marker-assisted selection of Chinese Dagu hens, but also provides a basic knowledge for further studies on SNPs detection on gremlin genes in other chicken breeds or any other animal species.

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