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Polymorphisms of pro-opiomelanocortin gene and the association with reproduction traits in chickens

Ke Liu[a], Haiyue Cao[a], Xinyang Dong[a], Honghua Liu[a], Yaya Wen[a], Haiguang Mao[a], Lei Lu[b], Zhaozheng Yin[a,*]

[a] *College of Animal Sciences, Zhejiang University, Zijingang Campus, Hangzhou 310058, China*
[b] *Ningbo Zhenning Animal Husbandry Co. Ltd, Ningbo 315000, China*

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ABSTRACT

Pro-opiomelanocortin (POMC) is a member of prohormone family and has important functions in stress response, skin pigmentation, thermoregulation and reproduction. In this study, the single nucleotide polymorphisms (SNPs) of *POMC* gene exons were detected by direct sequencing in 317 Zhenning yellow chickens. The sequencing results indicated there were seven mutation sites (g.1140C > T, g.1185 T > C, g.2085 T > C, g.3566A > C, g.3572 G > A, g.3594 G > A and g.3628 G > A) and all of these were synonymous. Furthermore, seven haplotypes were formed and sixteen diplotypes were obtained. The associations between the *POMC* gene polymorphisms or diplotypes and reproduction traits were also analyzed. The association analysis results indicated that the SNP of g.1140C > T was associated with egg production at 300 d of age (E300), fertilization rate (FR), hatching rate of hatching eggs (HEHR) and hatching rate of fertilized eggs (FEHR; $P < 0.05$). The SNP of g.3566A > C was associated with FR ($P < 0.05$), SNP of g.3594G > A was associated with egg weight at 300d of age (EW300; $P < 0.05$), and SNP of g.3628G > A was associated with HEHR and FEHR ($P < 0.01$), respectively. Furthermore, chickens with H2H3 diplotype had greater EW300 and FR than those with H1H7 and H3H4 diplotypes ($P < 0.05$). These results indicate the expression of the *POMC* gene had significant genotype effects on the reproduction traits of Zhenning yellow chickens, and that the H2H3 diplotype could be used as a potential genetic marker to improve the reproduction traits in chicken breeding.

1. Introduction

Reproduction performance is one of the most important traits in the poultry industry (Li et al., 2013). It, however, is difficult to improve reproduction by the conventional breeding methods in poultry breeding. Abdollahi-Arpanahi et al (2014) reported the predictability for hen house egg production (HHP) was 0.27 in broiler chickens, and Ghorbani et al (2013) calculated the direct heritability of reproduction traits in Fars native fowl, including egg weight at first day of laying (0.26), egg number (0.20), mean egg weight at 28, 30, 32 weeks of the laying period (0.59), and age at sexual maturity (0.45). In addition, to more accurate results, the study of potential candidate genes for QTL has a significant effect on economically important traits, particularly in poultry breeding, when there is a focus on locating QTLs associated with economic traits (Lamont et al., 1996; Ou et al., 2009). The study of candidate genes related to reproduction traits, therefore, is very important for improving the reproduction in chickens.

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[*] Corresponding author.
E-mail address: yzhzh@zju.edu.cn (Z. Yin).

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The hypothalamic-pituitary-gonadal (HPG) axis is thought to be a primary regulator of poultry reproductive endocrine systems and reproductive functions. There is, therefore, a greater focus on studies of the candidate genes related to chicken reproduction, basing on the hypothalamic-pituitary-gonadal (HPG) axis (Kuo et al., 2005). It had been increasingly evident that in most species stress-induced stimulation may have an important effect on animal reproduction, which is related to the inhibition of HPG axis activity by activation of the hypothalamic-pituitary-adrenal axis (Wingfield et al., 1995; Sapolsky et al., 2000). The POMC compound is produced and secreted by cells of the pituitary producing melanocyte-stimulating hormone (Takeuchi et al., 1999). It is a pro-hormone for many different peptide hormones, including adrenocorticotrophin (ACTH), melanocyte stimulating hormone (MSH), and β-endorphin, which are especially important for animal reproduction (Hadley and Haskellluevano, 2010). Both β-endorphin and adrenocorticotrophin (ACTH) have been reported to stimulate the secretion of testosterone from the testes of adult rabbits and guinea pigs indicating these hormones have important functions in animal reproduction (Juniewicz et al., 1988). Results of previous studies implicate the POMC neurons in modulation of reproductive functions of animals by regulating the metabolic processes (Wiesner et al., 1985; Backholer et al., 2009). In addition, results of other studies indicate melanocortin produced from POMC in the arcuate nucleus can inhibit the reproductive system by regulating the food intake of animals (Watanobe et al., 1999; Backholer et al., 2009, 2010; Xu et al., 2011a,b). These previous results provide strong evidence that it is important to understand regulation of the *POMC* gene and its association with animal reproduction. These results, therefore, further indicate that the *POMC* gene is a potential target gene that is important in regulating reproduction traits such as egg laying in chickens (Hagan and Brooks, 1998).

The chicken *POMC* gene was initially cloned from avian species and had the same structure as that of mammals (Takeuchi et al., 1999). The *POMC* gene is located on Chromosome 3 with a full length of 4525 bp containing three exons. Results of studies indicate polymorphisms of the *POMC* gene are associated with the production traits in broilers (Bai et al., 2012). The effects of these *POMC* gene polymorphisms on reproduction traits, however, have not been reported in chickens used for egg production. The objective of the present study, therefore, was to investigate the association between the SNPs in *POMC* gene and reproduction traits in chickens.

2. Materials and methods

The experiment was conducted in accordance with Chinese guidelines for animal welfare and approved by the animal welfare committee of the Animal Science College, Zhejiang University.

2.1. Animals and trait evaluated

A total of 317 Zhenning yellow chickens, a native breed belonging to the type of meat and egg dualpurpose birds in Zhejiang province of China were selected as experimental animals and fed breeder diets meeting the National Research Council nutrient requirements. These birds were from the Poultry breeding Center of the Ningbo Zhenning Animal Husbandry Co., Ltd in Zhejiang province. All the birds were from the same hatching batch, and the population structure was assessed using the principle components analysis and there were not any population stratification issues detected. All experimental birds were kept in single cages for laying record collection and had free access to water and feed. Reproduction traits of chickens that were evaluated include egg weight at 300 d of age (EW300), egg production at 300 d of age (E300), fertilization rate (FR), hatching rate of hatching eggs (HEHR) and hatching rate of fertilized eggs (FEHR). The eggs for production of the next generation of birds were collected for 7 consecutive days after 300 days of egg production (301 d to 307 d), then incubated with a relative humidity atmosphere of 65% to 75% at a temperature of 37 °C using the pedigree hatching method. The FR, HEHR and FEHR of each hen were calculated using the following equation: FR(%) = number of fertilized eggs/number of collected eggs × 100, HEHR(%) = number of hatching chickens/number of hatching eggs × 100, FEHR(%) = number of hatching chickens/number of fertilized eggs × 100. All the reproduction traits were determined using the Standards of The Poultry Production Performance Terms and Measurement Statistics Method (NY/T823-2004).

2.2. Isolation of genomic DNA, PCR amplification and DNA sequencing

The genomic DNA was extracted from the blood samples (2 ml blood) collected by wing vein sampling. The blood was placed in containers with 20% EDTA anticoagulant, and stored at −20 °C. Genomic DNA was extracted from frozen blood samples using the TIANGEN blood genomic DNA extraction kit (TIANGEN, Beijing).

The primer sequences based on the chicken *POMC* gene (GenBank NO. NC_006090.5) were designed using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA; Table 1). The PCR were conducted in a 50-μl volume, including 25 μl of 2× Taq PCR MasterMix, 2 μl of each primer, 2 μl of genomic DNA and double-distilled water. The cycling protocol was 5 min at 94 °C, 30 cycles at 94 °C for 30 s, 40 s at the annealing temperature, extending at 72 °C for 1 min, with a final extension at 72 °C for 10 min.

The PCR products were transferred to the Hangzhou Qingkezixi Biotech Co., Ltd (Hangzhou, China) for sequencing. The ABI 3730xl DNA Sequencer was used for sequencing using the Sanger method. Long fragments were sequenced using bi-directional sequencing and then assembled using the DNAStar software.

2.3. Statistical analysis

Descriptive statistics of the reproduction traits evaluated in this study were calculated using SPSS 21.0 (Table 2). The Excel program was used to calculate genotype frequency, allelic frequencies, heterozygosity (*He*), *Hardy–Weinberg* equilibrium, effective allele numbers (*Ne*) and polymorphism information content (*PIC*). The haplotypes were calculated using the HaploView software.

Table 1
Primer sequences used for amplification of *POMC* gene.

Primers	Sequences(5'-3')	Tm(°C)	Fragment(bp)
POMC1-F	TCTCCAAGGGCCATCCAGAG	59.5	1748
POMC1-R	TCCCTGATTTCCTGGTTTGTC	55.6	
POMC2-F	AGGCGGAGTGATACCTTGAGC	59.5	984
POMC2-R	CTTCCCTTCCTCTCGTTCCA	57.4	
POMC3-F	GGAGAGCATCCGCAAGTACG	59.5	
POMC3-R	TTGGGAGTAACCTATGCTGAAGT	56.0	1275
POMC4-F	GCTGCCAACCTCCATACCTAATAC	59.6	
POMC4-R	TGTCATGTAATCCGGGTTTAGG	55.8	1472

Due to the complexity of *POMC* gene, fragments were divided into four segments in the sequencing process and primers were designed respectively.

Table 2
Descriptive statistics of reproduction traits in chickens.

Traits	Sample size	Max	Min	Mean	Standard deviation
[1]EW300 (g)	317	65	40	53.3596	5.6527
[2]E300 (n)	317	94	75	86.9590	3.1121
[3]FR (%)	317	100	50	93.7398	13.5050
[4]HEHR (%)	317	100	25	84.4884	19.5170
[5]FEHR (%)	317	100	33.33	89.4157	16.2678

[1] EW300 = egg weight at 300d of age.
[2] E300 = egg production at 300d of age.
[3] FR = fertilization rate.
[4] HEHR = hatching rate of hatching eggs.
[5] FEHR = hatching rate of fertilized eggs.

Diplotypes were constructed on the basis of haplotypes in all of the experimental birds using PHASE 2.0 software. To value the amount of the genetic variation captured by each SNP, variance explained (VE) values were calculated by the formula: VE = (2*p*q*αˆ2)/additive_variance, where p and q are the allele frequency of minor and major alleles, respectively, and α is the amount of the variation that occurs as a result of the specific SNP. Associations between single polymorphisms or diplotypes and reproduction traits were analyzed using a General Linear Model (GLM) procedure utilizing SPSS 21.0. The LSD was used for the multiple comparisons. The model used was as follows:

$$Y_{ijkm} = \mu + G_i + A_j + M_k + S_m + e_{ijkm}$$

where Y_{ijkm} is the phenotypic value of traits, μ is the overall mean, G_i is the fixed effect of genotype or the diplotype, A_j is the fixed effect of age at the time of first egg production, M_k is the fixed effect of maternal environment, S_m is the sire effect and e_{ijkm} is random error.

3. Results

3.1. Genotype and allele frequencies

In this study, seven mutation sites were detected in Exon 2 (g.1140C > T and g.1185 T > C) and Exon 3 (g.2085 T > C, g.3566A > C, g.3572 G > A, g.3594 G > A and g.3628 G > A) the *POMC* gene, respectively. Each mutation site had three genotypes. No mutation, however, was detected in Exon 1. The data for genotypic frequency, *P*-values and the results using the *Hardy–Weinberg* equilibrium test are shown in Table 3. The results indicate that except for the g.3566A > C mutation site, the major alleles of each mutation site were all non-mutation alleles, and the major allele frequencies of each site were 0.85, 0.68, 0.88, 0.59, 0.76 and 0.72, respectively. Allele C was the major allele in the population at the g.3566A > C mutation site (0.58). The *P*-values indicated that all mutation sites were in agreement with the *Hardy–Weinberg* equilibrium ($P > 0.05$). The SNPs of g.1185 T > C, g.3566A > C, g.3572 G > A and g.3628 G > A were classified as having a moderate extent of polymorphism ($0.25 < PIC < 0.5$), while the rest of the mutation sites had a lesser extent of polymorphism ($PIC < 0.25$).

3.2. Amino acid sequence analysis

The amino acid changes resulting from these mutations are shown in Table 4. With all seven mutations, there was not a change in the amino acid sequence of the proteins of interest, thus, the amino acid sequence of the protein was not modified by the mutation (i.e., synonymous).

Table 3
Genotypes and alleles frequencies of the SNPs in *POMC* gene.

SNPs	Genotype frequencies(n)			Allelic frequencies		P-Value[4]	Genetic polymorphism		
	CC	CT	TT	C	T		*PIC*[1]	*He*[2]	*Ne*[3]
g.1140C > T	0.73(232)	0.24(77)	0.03(8)	0.85	0.15	0.87	0.22	0.28	1.3
	TT	TC	CC	T	C				
g.1185 T > C	0.49(156)	0.37(119)	0.13(42)	0.68	0.32	0.19	0.34	0.43	1.77
	TT	TC	CC	T	C				
g.2085 T > C	0.79(252)	0.18(56)	0.03(9)	0.88	0.12	0.52	0.18	0.21	1.26
	AA	AC	CC	A	C				
g.3566A > C	0.16(51)	0.52(165)	0.32(101)	0.42	0.58	0.50	0.37	0.49	1.95
	GG	GA	AA	G	A				
g.3572 G > A	0.36(114)	0.46(147)	0.18(56)	0.59	0.41	0.64	0.37	0.48	1.94
	GG	GA	AA	G	A				
g.3594 G > A	0.60(190)	0.32(102)	0.08(25)	0.76	0.24	0.35	0.11	0.11	1.13
	GG	GA	AA	G	A				
g.3628 G > A	0.54(172)	0.37(117)	0.09(28)	0.72	0.27	0.50	0.32	0.40	1.66

Numbers in parentheses indicated the number of individuals.

[1] *PIC*: polymorphism information content.

[2] *He*: gene heterozygosity.

[3] *Ne*: effective number of alleles.

[4] The test of *Hardy–Weinberg* equilibrium P-value > 0.05 suggested the population conforms to *Hardy–Weinberg* equilibrium.

Table 4
Identified SNPs within chicken *POMC* gene and the changes of amino acid structure.

SNPs	Mutation sites/ bp	Amino acid mutations
g.1140C > T	1140	Gly
g.1185 T > C	1185	Pro
g.2085 T > C	2085	Ala
g.3566A > C	3566	\
g.3572 G > A	3572	\
g.3594 G > A	3594	\
g.3628 G > A	3628	\

"\" in Table 4 represented that there were four sites (g.3566A > C, g.3572 G > A, g. 3594 G > A and g.3628 G > A) that didn't encode proteins because they were in non-coding regions.

3.3. Association of the POMC gene polymorphisms with reproduction traits

The data for the association between polymorphisms in the exon regions of the *POMC* gene and reproduction traits in chickens are shown in Table 5. The results indicate there are four mutation sites (g.1140C > T, g.3566A > C, g.3594 G > A and g.3628 G > A) that significantly affect reproduction traits. For the other three mutation sites (g.1185 T > C, g.2085 T > C and g.3572 G > A), there were no associations with the reproduction traits in chickens ($P > 0.05$). For the g.1140C > T SNP, birds with CC genotype had a greater E300 and FEHR than those with the TT genotype ($P < 0.05$), and birds with the CC and CT genotypes had a greater FR and HEHR than those with the TT genotype ($P < 0.05$). For the SNP of g.3566A > C, birds with the AC genotype had a greater FR than those with the CC genotype ($P < 0.05$). For the g.3594G > A locus, the GG genotype had a lesser EW300 than that of the GA genotype ($P < 0.05$). For the g.3628G > A locus, the GG genotype had a greater HEHR and FEHR than those of the GA genotype ($P < 0.05$).

The variance explained (VE) values are also shown in Table 5. For the SNP of g.1140C > T, the VE values for EW300, FR, HEHR and FEHR were 0.32%, 0.44%, 0.99% and 1.82%, respectively. For the SNP of g.3566A > C, the VE value for FR was 0.52%. For the SNP of g.3594 G > A, the VE value for EW300 was 0.66%. For the SNP of g.3628 G > A, the VE values for HERE and FEHR were 2.78% and 4.92%, respectively.

3.4. Analysis of haplotypes and diplotypes

The data resulting from the analysis of haplotypes and diplotypes of the *POMC* gene are included in Table 6. The results indicate that T-A-G-G, T-A-G-A, T-A-A-A, and T-C-G-A were the main haplotypes, accounting for 82.17% of the observations and formed the haplotype domain. Seven haplotypes were identified in 317 chickens based on four SNPs. Sixteen diplotypes were detected based on the seven haplotypes. To ensure that the analysis was accurate, five diplotypes with a frequency of less than 0.02 were not used in the further association analysis.

Table 5

Association analysis of SNPs in *POMC* gene with reproduction traits (mean ± SE).

SNPs	Genotypes(*n*)	[1]EW300(g)	[2]E300(*n*)	[3]FR(%)	[4]HEHR(%)	[5]FEHR(%)
g.1140C > T	CC (232)	53.47 ± 0.370	87.02 ± 0.207^{a}	93.96 ± 1.00^{a}	85.27 ± 1.57^{a}	90.45 ± 1.42^{a}
	CT (77)	53.38 ± 0.650	87.01 ± 0.325^{ab}	94.59 ± 1.83^{a}	84.20 ± 2.88^{a}	87.99 ± 2.72^{ab}
	TT (8)	50.00 ± 1.890	84.75 ± 1.411^{b}	79.17 ± 12.50^{b}	64.58 ± 11.11^{b}	72.92 ± 12.57^{b}
P-value		0.088	0.043	0.011	0.031	0.032
V_A[6]		0.0868	0.0308	0.8057	3.7846	4.8286
VE[7]		0.0027	0.0032	0.0044	0.0099	0.0182
g.1185 T > C	TT (156)	53.08 ± 0.440	86.92 ± 0.257	91.99 ± 1.52	82.05 ± 2.19	87.87 ± 2.08
	TC (119)	53.78 ± 0.560	86.87 ± 0.274	95.38 ± 1.20	86.76 ± 2.06	90.69 ± 1.85
	CC (42)	53.21 ± 0.759	87.36 ± 0.483	95.63 ± 1.89	87.10 ± 2.79	91.47 ± 2.45
P-value		0.307	0.380	0.087	0.115	0.311
V_A		0.0375	0.0066	2.4759	4.7727	2.0440
VE		0.0012	0.0007	0.0136	0.0125	0.0077
g.2085 T > C	TT (252)	53.29 ± 0.359	86.88 ± 0.207	94.11 ± 0.99	85.45 ± 1.46	90.41 ± 1.33
	TC (56)	53.75 ± 0.746	87.45 ± 0.286	91.67 ± 2.57	79.76 ± 4.08	84.67 ± 3.90
	CC (9)	52.78 ± 1.690	86.22 ± 1.115	96.30 ± 3.70	87.04 ± 6.68	90.74 ± 6.28
P-value		0.586	0.216	0.310	0.117	0.088
V_A		0.0176	0.0265	0.5350	3.6087	3.9466
VE		0.0006	0.0027	0.0029	0.0095	0.0149
g.3566A > C	AA (51)	52.45 ± 0.746	86.78 ± 0.523	92.81 ± 2.65	81.70 ± 4.16	86.93 ± 4.05
	AC (165)	53.55 ± 0.428	87.09 ± 0.237	95.61 ± 0.95^{a}	85.51 ± 1.77	89.65 ± 1.63
	CC (101)	53.51 ± 0.603	86.83 ± 0.288	91.17 ± 1.99^{b}	84.24 ± 2.45	90.26 ± 2.22
P-value		0.228	0.511	0.031	0.335	0.395
V_A		0.0938	0.0002	0.9538	0.3634	1.0907
VE		0.0097	0.0001	0.0052	0.0010	0.0041
g.3572 G > A	GG (114)	53.68 ± 0.568	86.88 ± 0.279	91.59 ± 1.806	85.23 ± 2.227	91.15 ± 1.987
	GA (147)	53.50 ± 0.451	87.04 ± 0.246	95.52 ± 1.025	85.37 ± 1.923	89.57 ± 1.766
	AA (56)	52.32 ± 0.697	86.91 ± 0.496	93.45 ± 2.428	80.65 ± 3.864	85.42 ± 3.800
P-value		0.140	0.675	0.053	0.222	0.123
V_A		0.1475	0.0007	0.9158	1.4545	2.9396
VE		0.0046	0.0001	0.0050	0.0038	0.0111
g.3594 G > A	GG (190)	52.87 ± 0.399^{b}	87.07 ± 0.217	93.95 ± 1.177	83.90 ± 1.829	88.68 ± 1.72
	GA (102)	54.46 ± 0.557^{a}	86.75 ± 0.342	93.46 ± 1.645	85.54 ± 2.424	90.28 ± 2.222
	AA (25)	52.60 ± 1.295^{ab}	86.96 ± 0.511	93.33 ± 3.191	84.67 ± 3.958	91.33 ± 3.209
P-value		0.022	0.413	0.810	0.588	0.570
V_A		0.2118	0.0136	0.0594	0.3927	0.7862
VE		0.0066	0.0014	0.0003	0.0010	0.0030
g.3628 G > A	GG (172)	53.40 ± 0.438	87.00 ± 0.222	94.72 ± 1.028	88.08 ± 1.527^{A}	93.17 ± 1.274^{A}
	GA (117)	53.38 ± 0.511	86.97 ± 0.313	91.52 ± 1.884	79.56 ± 2.712^{B}	84.69 ± 2.642^{B}
	AA (28)	53.04 ± 1.101	86.68 ± 0.602	97.02 ± 2.109	83.04 ± 4.705^{AB}	86.01 ± 4.557^{AB}
P-value		0.752	0.614	0.101	0.004	0.002
V_A		0.0045	0.0040	0.2535	10.5942	13.0117
VE		0.0001	0.0004	0.0014	0.0278	0.0492

Numbers in parentheses indicated the number of individuals.

Means with the different uppercase superscripts were different ($P < 0.01$), and the means with different lowercase superscripts were different ($P < 0.05$); Means with the same superscripts were not different ($P > 0.05$).

Means without superscripts were not different ($P > 0$.

[1] EW300 = egg weight at 300d of age.

[2] E300 =egg production at 300d of age.

[3] FR = fertilization rate.

[4] HEHR = hatching rate of hatching eggs.

[5] FEHR = hatching rate of fertilized eggs.

[6] V_A = additive genetic variance.

[7] VE = variance explained, VE = (2*p*q*αˆ2)/additive_variance, where p and q are the allele frequency of minor and major alleles, respectively, and α is the amount of the variation that occurs as a result of the specific SNP.

3.5. Associations of diplotypes of POMC gene with reproduction traits in chickens

The data from the association analysis are reported in the Table 7. The results indicated that chickens with H2H3 diplotype had a greater EW300 and FR than those with H1H7 diplotype ($P < 0.05$) and H3H4 diplotype ($P < 0.05$) respectively. Furthermore, the birds with the H3H4 diplotype had the least FR among all diplotypes ($P < 0.01$). Birds with the H1H5 and H2H2 diploytpes had a greater FEHR than those with H4H5 diplotype ($P < 0.05$).

Table 6

Haplotypes based on the four SNPs of *POMC* gene.

Haplotypes	g.2085 T > C	g.3566 A > C	g.3572 G > A	g.3594 G > A
H1	T	A	G	G
H2	T	A	G	A
H3	T	A	A	A
H4	T	C	G	G
H5	T	C	G	A
H6	C	A	A	A
H7	C	C	G	G

Table 7

Association analysis of *POMC* gene diplotypes with reproduction traits in chickens.

Diplotypes	Frequency	EW300 (g)	E300 (*n*)	FR (%)	HEHR (%)	FEHR (%)
H1H3	0.0631	53.00 ± 1.33	86.55 ± 0.84	94.58 ± 3.10^{A}	87.92 ± 5.11	92.50 ± 4.27
H1H5	0.1640	53.56 ± 0.81	87.17 ± 0.38	95.03 ± 1.82^{A}	89.58 ± 2.39	94.55 ± 1.86^{a}
H1H7	0.1483	52.34 ± 0.79^{b}	86.60 ± 0.55	92.91 ± 2.81^{A}	80.85 ± 4.45	85.82 ± 4.36
H2H2	0.0410	55.00 ± 1.50	88.08 ± 0.98	94.87 ± 3.47^{A}	92.31 ± 4.05	97.44 ± 2.56^{a}
H2H3	0.0536	55.59 ± 1.71^{a}	86.24 ± 0.59	90.20 ± 6.24^{ABc}	88.24 ± 6.35	92.16 ± 6.08
H2H5	0.1893	53.92 ± 0.70	86.62 ± 0.47	94.72 ± 1.79^{A}	83.75 ± 3.09	88.75 ± 2.82
H3H3	0.0789	52.60 ± 1.30	86.96 ± 0.51	93.33 ± 3.19^{A}	84.67 ± 3.96	91.33 ± 3.21
H3H4	0.0379	53.33 ± 1.88	86.75 ± 0.74	77.78 ± 9.25^{Bd}	75.00 ± 9.06	88.89 ± 8.54
H3H5	0.0315	56.00 ± 1.63	87.60 ± 0.86	88.33 ± 6.11	80.00 ± 11.33	85.00 ± 10.67
H3H7	0.0473	52.33 ± 1.18	87.80 ± 0.60	95.56 ± 3.03^{A}	82.22 ± 5.26	86.67 ± 5.19
H4H5	0.1041	52.88 ± 0.92	87.58 ± 0.31	97.47 ± 1.43^{A}	80.81 ± 5.17	82.58 ± 5.00^{b}
P-value		0.045	0.108	0.001	0.068	0.020

Means in the same column with the different uppercase superscripts were different ($P < 0.01$), and means with different lowercase superscripts were different ($P < 0.05$); Means with the same letters were not different ($P > 0.05$);
Means without superscripts were not different ($P > 0.05$).

[1]EW300 = egg weight at 300d of age; [2]E300 = egg production at 300d of age; [3]FR = fertilization rate; [4]HEHR = hatching rate of hatching eggs; [5]FEHR = hatching rate of fertilized eggs.

4. Discussion

In recent years, QTLs associated with reproduction traits have been found to be important in modern animal breeding and findings in many studies have resulted in correlations between markers for candidate genes and reproduction traits in chickens (Cui et al., 2006; Xu et al., 2011a,b). Multiple genes regulate most of reproduction traits in chickens. The use of molecular marker assisted selection techniques, therefore, is an effective way to improve these economic traits within a relatively short period of time compared to using traditional quantitative approaches and result in advancements in chicken breeding.

Results of studies indicate there is greatest expression of the *POMC* gene in the brain, hypothalamus, pituitary and ovary as compared with other tissues (Benjannet et al., 1991; Sharma et al., 2008; Yoshihara et al., 2011; Noy et al., 2017). Mcmillen et al (1988) reported that there was a greater abundance of *POMC* mRNA transcript in the sheep fetus using the Northern blot method. In addition, the abundance of *POMC* mRNA changed from pregnancy to lactation indicating the products from *POMC* gene expression affected reproduction (Mann et al., 1997).

Results of previous studies indicate there is a close association among processes regulating energy storage, the melanocortin system and the reproduction system (Moberg, 1985; Backholer et al., 2010). The *POMC* gene was, therefore, thought to have an important function in regulating energy balance and reproduction (Hill et al., 2008). There, however, are few studies focused on genetic effects controlling reproduction traits as related to *POMC* gene expression in chickens. The present study, therefore, is the first where there is analysis of the association between *POMC* gene polymorphism and reproduction traits in chickens.

In the present study, seven mutation sites were screened in the exons of the *POMC* gene, and all the mutations were in agreement with *Hardy–Weinberg* equilibrium. In addition, the polymorphic information content of each mutation site was evaluated. It was determined that g.1185 T > C, g. 3566A > C, g.3572 G > A and g.3628 G > A had moderate polymorphism content, while the other loci had very few polymorphism content. All the seven mutations were synonymous, however, results of many studies indicate these silent mutations also have important effects on protein function by changing the stability of the mRNA (Ren et al., 2014; Wang et al., 2014).

In the present study, there was analysis of the polymorphisms of the *POMC* gene and its relationship with the reproduction traits of Zhenning yellow chickens. In the previous study, thirteen polymorphisms of the *POMC* gene were identified in all 825 broilers of the pedigree line, and a missense mutation (Pro to Leu) was associated with greater BW in females (Sharma et al., 2008). In the present study, the SNP of g.1140C > T in Exon2 was identified and had a significant association with FR, HEHR and FEHR in chickens. The SNP at the g.3628 G > A locus, was associated with a greater HEHR and FEHR in the birds with the GG genotype than those with the GA genotype ($P < 0.05$). Furthermore, for the SNP at the g.3566A > C locus, individuals with the AC genotype had a greater FR than those with the CC genotype ($P < 0.05$). At the g.3594G > A locus, the EW300 of birds with GG genotype was less than that of birds with the GA genotype ($P < 0.05$). The values for the magnitude of variance explained (VE) by these polymorphisms, however, were relatively less in the present study for these SNPs.

Diplotypes were associated with EW300 in the present study. The results indicated that the values for EW300 of birds with the H2H3 diplotype were greater than those with H1H7 diplotype ($P < 0.05$). The H2H3, therefore, was regarded as the diplotype having the greatest effect on reproduction traits in chickens. The birds with the H3H4 diplotype had the least FR among the birds with the different diplotypes and had no significant effects on the other reproduction traits. As a result, this diplotype could be regarded as being detrimental for reproduction trait expression in chickens, therefore, it could be deleted during the cultivation of new varieties.

In conclusion, the results of the present study indicated that polymorphisms of the *POMC* gene had potential effects on reproduction traits in chickens. Futhermore, the H2H3 diplotype could be used as a potential genetic marker to improve reproduction traits in chicken breeding.

Declaration of Competing Interest

All authors declare no conflicts of interest.

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