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Variants of pri-miR-26a-5p polymorphisms are associated with values for chicken egg production variables and affects abundance of mature miRNA

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ABSTRACT

MicroRNAs (miRNAs) are a class of endogenous non-coding RNAs that have an important role in post-transcriptional regulation. Previous analysis of the chicken ovary transcriptome indicated there was a differential abundance of miR-26a-5p in sexually mature and immature hens. In this study, ten single nucleotide polymorphism (SNP) sites in miR-26a-5p primary transcripts (pri-miR-26a-5p) were identified using Sanger sequencing on PCR products in three chicken breeds, among which, the rs316365438(C > T)SNP was associated with age at first egg (AFE) and total number of eggs at 32 weeks (E32). The hens with the TT genotype of the rs316365438(C > T) SNP had an earlier AFE and greater E32 than those with CC and CT genotypes. Due to the presence of linkage disequilibrium (LD) in SNPs, there were only two dominant haplotypes. The two dominant haplotypes with different free-energy and secondary structure were identified and the overexpression vectors were constructed. Overexpression of mature miR-26a-5p from TCT ACGCACG haplotype was greater than that from the TTTATATACA haplotype. In summary, the polymorphisms of pri-miR-26a-5p are associated with chicken egg production traits and affect the transcript abundance of the mature miR-26a-5p.

1. Introduction

The microRNAs are evolutionarily conserved single-stranded RNA molecules that are approximately 22 nucleotides in length, and function as important post-transcriptional regulators in various organisms and tissues (Bartel, 2004). The miRNAs are involved in a broad range of biological processes, such as cell proliferation (Jiang et al., 2015), cell differentiation (Dai et al., 2016), cell apoptosis (Tu et al., 2014; Liu et al., 2014), cholesterol homeostasis (Najafi-Shoushtari et al., 2010) and tumorigenesis (Yao et al., 2017). Mature miRNAs are generated as a result of three processes. First, the long primary transcripts (pri-miRNA) is processed into precursor miRNA (pre-miRNA) as a result of functions of the Drosha/DGCR8 complex in the nucleus (Lee et al., 2003; Denli et al., 2004). The pre-miRNA is then exported from the nucleus to the cytoplasm as a result of the functions of Exportin-5, and subsequently pre-miRNAs are recognized and cleaved as a result of functions of the RNase III Dicer to generate mature miRNA (Ketting et al., 2001; Yi

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et al., 2003; Bohnsack et al., 2004). The inconsistencies among primary, precursor, and mature miRNA abundances indicate that the abundances of mature miRNAs are regulated during miRNA processing (Slezak-Prochazka et al., 2010). Apart from this canonical biogenesis pathway, some intronic miRNAs precursors can generate mature miRNAs through an alternative pathway without Drosha-mediated cleavage, this pathway is termed the "mirtron pathway" (Ruby et al., 2007).

Single nucleotide polymorphisms (SNPs) in pri-miRNA, pre-miRNA and mature miRNA are associated with the abundances of mature miRNA and may contribute to the evolution of miRNAs with new functions (Duan et al., 2007; Sun et al., 2009). There have been reports that the SNP density of pre-miRNAs was less than that of flanking regions, while the presence of SNPs in miRNA was rare (Saunders et al., 2007). The SNPs affect the function of miRNA genes by regulating the transcription of the primary transcript, pri-miRNA and pre-miRNA processing, and in miRNA-mRNA interactions (Ryan et al., 2010). There was an initial study where miRNA-related SNPs were reported to affect phenotype and as a result mutation in the miR-189 binding site of *SLITRK1* was associated with Tourette's syndrome (Abelson et al., 2005).

Results of another previous study indicate the abundance of miR-26a-5p transcript changed in chicken ovaries during sexual maturation (Kang et al., 2013). In a further study miR-26a-5p regulated *TNRC6A* gene expression and modulated theca cell proliferation in chicken ovarian follicles (Kang et al., 2017). It, however, is unclear whether the SNPs of miR-26a-5p affect abundance of miRNA transcripts and chicken reproductive performance. In the present study, there was assessment of SNPs in pri-miR-26a-5p and analysis of associations with values for egg production variables. There was also investigation of whether those SNPs could alter the abundance of mature miRNA transcripts.

2. Materials and methods

2.1. Birds, sampling and DNA extraction

White Recessive Rock (n = 315), Jining Bairi (n = 50), and Xinyang Brown (n = 27) chickens, randomly selected from the respective breeding populations, were used for the polymorphism study. The White Recessive Rock chickens were also used for a genotype-trait association analysis. The White Recessive Rock chickens, were housed individually in laying batteries with free access to feed and water, and were exposed to a 16L: 8D photoperiod. The laying pattern of each hen was recorded daily, and values for egg production variables, including age at first oviposition (AFE), total number of eggs at 32 weeks of laying (E32) and total number of eggs at 48 weeks of laying (E48) were collected. Sampling was performed by collecting 1 ml of blood per hen from the wing vein. The birds were managed and cared for in ways consistent with the guidelines of the Animal Care and Use Committee of Shangdong Agricultural University. Genomic DNA was extracted from blood samples using a TIANamp Genomic DNA kit (TIANGEN Biotech Co., Ltd, Beijing, China). The DNA samples were assessed using 1% agarose gel electrophoresis, and OD260/280 ratios of 1.8 were determined.

2.2. Polymorphism detection and genotyping

The chicken miR-26a-5p is located in Intron 5 of the CTD small phosphatase like-205 (CTDSPL-205) host gene on Chromosome 2. To confirm the polymorphism sites in pri-miR-26a-5p, a pair of polymerase chain reaction (PCR) sequencing primers of poly-miR-26a-F/R (Table 1) were designed using the public chicken sequence (GenBank accession No: NC_006089.4). The primers were subsequently used to amplify a 672-bp fragment containing the miR-26a-5p precursor sequence (pre- miR-26a-5p). The PCR products were sent to Biosune (Shanghai, China) for sequencing with ABI3730XL as DNA analyzer (Applied Biosystems). Sequences were aligned using the DNAMAN program (https://dnaman.software.informer.com/8.0/), and the genotypes were determined using the ChromasPro program (https://chromaspro.software.informer.com/2.0/).

2.3. Secondary structure prediction

The most stable secondary structure of pri-miR-26a-5p with minimal free energy was predicted by the M-fold (Zuker, 2003) program. The absolute difference in free energy for pri-miR-26a-5p with different haplotypes was used as the parameter for assessing of the impact of the secondary structure on pri-miR-26a-5p.

Table 1
Primers used for polymorphism analyses and plasmids construction and qPCR of miR-26a-5p.

Primer name	Primer sequence (5′-3′)	Annealing temperature
Poly-miR-26a-F	ATCAGCCCATCACCACCAT	60 °C
Poly-miR-26a-R	TACCTCAAAGCAGTCCCAGC	60 °C
miR-26a-KpnI-F	GGGGTACCATCAGCCCATCACCACCAT	
miR-26a-XhoI- R	CCGCTCGAGTACCTCAAAGCAGTCCCAGC	
5S rRNA	GGTTAGTACTTGGATGGGAGACTGCCT	61 °C
mature-miR-26a	GGGCGTTCAAGTAATCCAGGATAGGC	60 °C

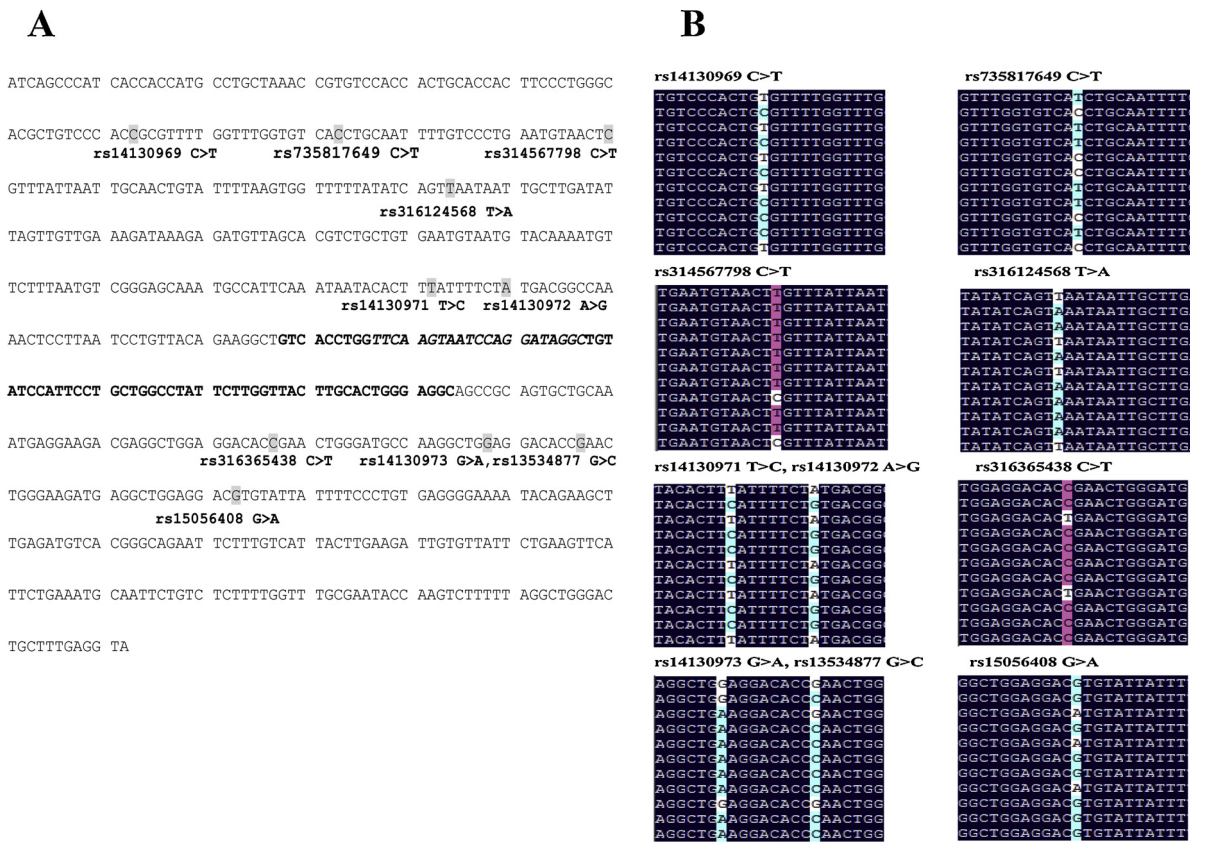

Fig. 1. Location and genotyping of ten SNPs in pri-miR-26a-5p. (A); Position of ten SNPs relative to pre-miR-26a-5p, the blackbody represents the sequence of pre-miR-26a-5p, and italics represent mature miR-26a-5p; (B) Sequencing alignment of ten SNPs among different hens.

2.4. Plasmid constructs

To investigate the effect of different haplotypes on the abundance of mature miR-26a transcripts, two major gene overexpression vector plasmids were constructed based on the haplotype analysis. The 672-bp genomic fragment encompassing the pre-miR-26a-5p sequence (GenBank accession No: NC_006089.4) and its 327-bp 5′ and 268-bp 3′ flanking regions were amplified by using primer pairs of miR-26a-KpnI-F/XhoI-R (Table 1). Then, the PCR products were cloned into the pcDNA3.1 (+) vector (Invitrogen). All constructs were verified using DNA sequencing.

2.5. Cell culture and transfection

Granulosa cells and theca cell separation methods were conducted as previously described by Gilbert et al., (1977). Briefly, the granulosa layer was isolated and then gently agitated in a flask with 0.2% (w/v) collagenase II (Gibco) at 37 °C for 8 min. Similarly, the theca layer was dissected from surrounding tissues and placed in 0.2% (w/v) collagenase II at 37 °C for 30 min with gentle agitation in a flask. After centrifugation, the cells were suspended in M199 (HyClone) culture medium supplemented with 10% (v/v) fetal bovine serum (HyClone) and 1% (v/v) penicillin/streptomycin (Gibco). Cells were subsequently seeded in 24-well culture plates at a density of 1×10^6/well. The number of viable cells (90%) was estimated using Trypan blue. Cells were cultured at 38.5 °C in a water-saturated atmosphere of 95% air with 5% CO_2. The two constructed plasmids of pri-miR-26a were transfected into cells using Lipofectamine™ LTX and PLUSTM Reagent (Invitrogen). An empty pcDNA3.1 plasmid was used as the negative control. Transfection efficiency was assessed by transfecting the pEGFP-C1 vector. Each sample was transfected in triplicate and the experiment was repeated at least three times. At 48 h after transfection, the cells were collected using lysate in a miRcute miRNA isolation kit (Tiangen).

2.6. Detection of abundance mature miR-26a-5p transcript

Total RNA from the cultured cells was extracted. The quantity of RNA was determined using a BioPhotometer Plus (Eppendorf, Germany), and its integrity was assessed using 1.5% agarose gel electrophoresis. The RNA samples were reverse-transcribed into cDNA using a Mir-X miRNA First-Strand Synthesis Kit (TaKaRa). The reverse transcriptase reaction components consisted of 5 μL of

Table 2
Genotypic and allelic frequencies of chicken pri-miR-26a SNPs and Hardy-Weinberg equilibrium test in different hen populations.

Sites	Breeds	Genotype frequency			Allele frequency		*P*- value
Rs14130969		CC	CT	TT	C	T	
C > T	WRR(n = 315)	0.003	0.098	0.899	0.052	0.948	0.878
	JB (n = 50)	0	0.160	0.840	0.080	0.920	0.539
	XB (n = 27)	0	0	1	0	1	–
Rs735817649		CC	CT	TT	C	T	
C > T	WRR (n = 315)	0.473	0.460	0.667	0.703	0.297	0.068
	JB (n = 50)	0.700	0.160	0.140	0.780	0.220	0.00016
	XB (n = 27)	1	0	0	1	0	–
Rs314567798		CC	CT	TT	C	T	
C > T	WRR (n = 315)	0.006	0.216	0.778	0.114	0.886	0.239
	JB (n = 50)	0.080	0.220	0.700	0.190	0.810	0.044
	XB (n = 27)	0	0	1	0	1	–
Rs316124568		TT	AT	AA	T	A	
T > A	WRR (n = 315)	0.476	0.403	0.549	0.249	0.751	0.169
	JB (n = 50)	0.100	0.260	0.640	0.230	0.770	0.060
	XB (n = 27)	0	0	1	0	1	–
Rs14130971		TT	CT	CC	T	C	
T > C	WRR (n = 315)	0.222	0.556	0.222	0.500	0.500	0.049
	JB (n = 50)	0.260	0.620	0.120	0.570	0.430	0.061
	XB (n = 27)	0.04	0.444	0.518	0.259	0.741	0.414
Rs14130972		AA	AG	GG	A	G	
A > G	WRR (n = 315)	0.190	0.524	0.286	0.452	0.548	0.288
	JB (n = 50)	0.120	0.620	0.260	0.430	0.570	0.061
	XB (n = 27)	0	0.370	0.630	0.185	0.815	0.237
Rs316365438		CC	CT	TT	C	T	
C > T	WRR (n = 315)	0.457	0.479	0.064	0.847	0.153	0.077
	JB (n = 50)	0.820	0.180	0	0.910	0.090	0.484
	XB (n = 27)	1	0	0	1	0	–
Rs14130973		GG	AG	AA	G	A	
G > A	WRR (n = 315)	0.048	0.248	0.704	0.847	0.153	0.023
	JB (n = 50)	0.180	0.360	0.460	0.360	0.640	0.122
	XB (n = 27)	0	0	1	0	1	–
Rs13534877		GG	CG	CC	G	C	
G > C	WRR (n = 315)	0.060	0.235	0.705	0.847	0.153	0.00048
	JB (n = 50)	0.120	0.500	0.120	0.370	0.630	0.608
	XB (n = 27)	0	0.407	0.593	0.204	0.796	0.184
Rs15056408		GG	AG	AA	G	A	
G > A	WRR (n = 315)	0.330	0597	0.073	0.847	0.153	0.00048
	JB (n = 50)	0.720	0.260)	0.020	0.850	0.150	0.890
	XB (n = 27)	1	0	0	1	0	–

Note: WRR (White Recessive Rock chicken), JB (Jining Bairi chicken), XB (Xinyang Brown chicken); $P < 0.05$ indicate that genotype distribution was not in agreement with the Hardy-Weiberg equilibrium (HWE).

2× miRNA Reaction Buffer Mix, 1.25 μL of miRNA PrimeScript® RT Enzyme Mix, 3.75 μL of total RNA (2 μg/μL) and as much as10 μL of RNase-free ddH_2O. The RT-PCR program was conducted at 37 °C for 60 min and 85 °C for 5 s. Real-time quantitative PCR (qPCR) was performed using an Mx3000P™ SYBR® Green Real-time quantitative PCR Analyzer (Stratagene, USA). 5S rRNA (GenBank accession No: NC_006096) was used as an internal control, and all primer sequences are listed in Table-1. The reaction components included 10 μL of SYBR® Premix ExTaq™ II (2×), 0.8 μL of PCR Forward Primer (10 μM), 0.8 μL of Uni-miR qPCR Primer (10 μM), 0.4 μL of ROX Reference Dye II (50×), 2 μL of cDNA, and up to 20 μL of ddH_2O. The reaction mixtures were incubated in a 96-well plate at 95 °C for 30 s followed by 35 cycles at 95 °C for 5 s, 60 °C for 40 s, 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. All reactions were conducted in triplicate. The qPCR Analyzer automatically generated the Ct values of the target gene and the housekeeping gene, and the relative mRNA transcript abundance was calculated using the method of $2^{-\Delta\Delta Ct}$.

2.7. Statistical analysis

The SHEsis software (http://analysis.bio-x.cn) was used to analyze the linkage disequilibrium (LD) between several SNPs in one gene. The Lewontin D′ statistic > 0.8 is used to ascertain a sufficiently strong LD (Ardlie et al., 2002; Grunau et al., 2006). The genotype and allelic frequencies determined using the Hardy-Weinberg equilibrium X^2 test were calculated with the Population Genetic Analyses software (http://www.marksgeneticsoftware.net/tfpga.htm). The PHASE v2.0 program (Stephens et al., 2001) was applied to construct haplotypes in chicken populations. The associations of pri-miR-26a-5p genotypes with egg production traits including AFE, E32 and E48, were analyzed in White Recessive Rock hen populations that were from the same chicken farm using the General Linear Model of SAS (version 9.2; Cary, NC). The linear model is represented as follows: $Y_{ij} = \mu + G_i + e_{ij}$, where Y_{ij} is the phenotypic value of traits, $\mu$is the population mean, G_i is the fixed effect of genotype, and e_{ij} is the random error effect. For qPCR

Table 3
Association analyses of SNPs in Pri-miR-26a-5p with age of the first egg (AFE) and egg number at 32 and 48 weeks (E32, E48) in White Recessive Rock chickens (n = 315).

Sites	Traits	genotype			*P*-value
Rs14130969		CC	CT	TT	
C > T	AFE	182 + 0.000	178.677 + 1.362	177.753 + 0.524	0.763
	E32	29 + 0.000	29.419 + 1.023	31.272 + 0.421	0.356
	E48	119 + 0.000	107.935 + 1.88	110.442 + 0.527	0.215
Rs735817649		CC	CT	TT	
C > T	AFE	177.396 + 0.699	178.710 + 0.745	175.238 + 1.567	0.155
Rs314567798	E32	31.470 + 0.560	30.372 + 0.602	33.238 + 1.144	0.136
C > T	E48	111.168 + 0.667	109.317 + 0.824	109.762 + 1.874	0.210
Rs316124568	AFE	CC	CT	TT	0.273
T > A	E32	187.500 + 5.500	178.118 + 1.160	177.706 + 0.539	0.328
Rs14130971	E48	24.500 + 4.500	30.632 + 0.913	31.261 + 0.434	0.959
T > C	AFE	110.500 + 8.500	109.941 + 1.748	110.298 + 0.569	0.147
Rs14130972	E32	TT	AT	AA	0.196
A > G	E48	175.600 + 2.576	178.945 + 0.784	177.254 + 0.640	0.386
Rs316365438	AFE	31.267 + 2.417	30.228 + 0.637	31.694 + 0.496	0.688
C > T	E32	112.800 + 3.114	109.606 + 0.912	110.451 + 0.587	0.779
Rs14130973	E48	TT	CT	CC	0.671
G > A	AFE	178.300 + 1.072	177.983 + 0.657	177.100 + 1.009	0.686
Rs13534877	E32	30.586 + 0.897	31.166 + 0.526	31.371 + 0.772	0.781
G > C	E48	109.543 + 1.415	110.217 + 0.637	110.914 + 415	0.845
Rs15056408	AFE	AA	AG	GG	0.034
G > A	E32	178.283 + 1.205	178.060 + 0.685	177.191 + 0.849	0.045
	E48	30.700 + 1.031	31.006 + 0.542	31.483 + 0.654	0.328
	AFE	109.650 + 1.621	110.271 + 0.658	110.517 + 0.763	0.343
	E32	CC	CT	TT	0.369
	E48	177.076 + 0.706^{ab}	179.033 + 0.722^{a}	174.600 + 1.700^{b}	0.866
	AFE	31.549 + 0.565^{ab}	30.258 + 0.580^{b}	33.950 + 1.280^{a}	0.055
	E32	110.035 + 0.672	109.457 + 0.807	110.150 + 2.003	0.133
	E48	GG	AG	AA	0.054
	AFE	177.200 + 2.118	179.103 + 1.110	177.464 + 0.556	0.347
	E32	32.067 + 1.876	30.154 + 0.888	31.342 + 0.443	0.585
	E48	111.200 + 2.442	109.885 + 1.838	110.275 + 0.572	0.704
		GG	CG	CC	
		173.895 + 1.636	179.203 + 1.018	177.748 + 0.584	
		32.895 + 1.982	29.824 + 0.755	31.347 + 0.464	
		114.421 + 2.663	110.324 + 0.560	108.838 + 1.174	
		GG	AG	AA	
		176.952 + 0.853	178.441 + 0.639	177.174 + 1.643	
		31.413 + 0.690	30.777 + 0.518	32.087 + 1.109	
		110.779 + 0.812	109.872 + 0.697	110.565 + 1.771	

Note: Means with different lowercase letters within the same row are significantly different at $P < 0.05$.

analysis, differences between the experimental groups were evaluated by one way analysis of variance (ANOVA), followed by use of the Duncan's multiple range test for performing the General Linear Model procedure of SAS (SAS version 9.2; Cary, NC). All data are presented as the mean + SEM and the differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Identification and genotyping of miR-26a-5p SNPs

The mutations in the primary region of miR-26a-5p were detected in three different chicken breeds by directly sequencing PCR products, and ten single nucleotide polymorphisms (SNPs) were identified (Fig.1 and S1 Table): rs14130969 (C > T), rs735817649 (C > T), rs314567798 (C > T), rs316124568 (T > A), rs14130971 (T > C), rs14130972 (A > G), rs316365438 (C > T), rs14130973 (G > A), rs13534877 (G > C), rs15056408 (G > A). The allele and genotype frequencies in different chicken populations are shown in Table 2.

3.2. Association of polymorphisms with values for egg production variables

To investigate the effect of the ten SNPs on values for egg production variables, association analyses of these SNPs with AFE, E32 and E48 were performed for the White Recessive Rock population. Among the ten SNPs, only rs316365438 (C > T) was associated with the chicken AFE and E32 ($P < 0.05$; Table 3). Hens with the TT genotype had an earlier AFE and a greater E32 compared to the other genotypes, but there was no difference between the CC and CT genotypes.

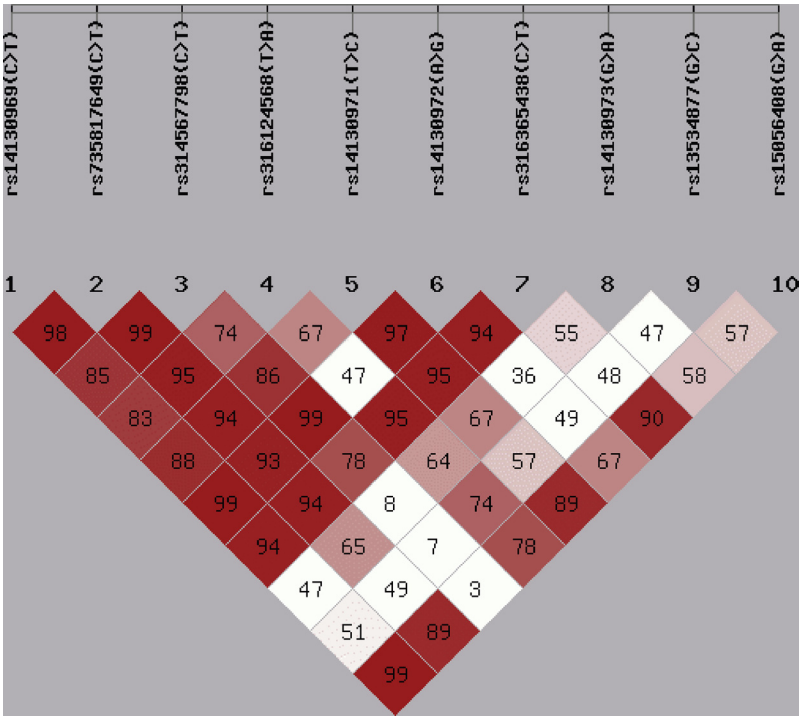

Fig. 2. Linkage disequilibrium (LD) test results using SHEsis software based on the value D′; Normalized LD is depicted as D′, and values of D′ range from 0% to 100%.

Table 4
Haplotypes based on the ten SNPs and its frequency.

Haplotypes	Frequency
TCTACGCACG	0. 361
TTTATATACA	0. 236
TCTTTACGGG	0. 069
CCCTCGCAGG	0. 026
TCTTTACAGG	0. 025
TCTTTACACG	0. 018
TCTACGCACA	0. 016
TCTTTACAGG	0. 015
TCCACGCACG	0. 012
TCTATGCGCA	0. 011
TCTACGCGCG	0. 011
CCCTCGCACG	0.010

3.3. Linkage disequilibrium analysis

Some miRNA SNPs might be in linkage disequilibrium and be inherited together. The linkage disequilibrium was analyzed between these SNPs using SHEsis software. Considering the linkage disequilibrium conditions (D′ statistic > 0.8), rs14130969 and rs735817649, rs14130972, rs316365438, rs15056408; rs314567798 and rs14130972, rs316365438; rs735817649 and rs314567798 had strong linkage disequilibria (Fig. 2).

3.4. Haplotype construction and secondary structure prediction

In total, twelve haplotypes from the ten SNPs were constructed and are presented in Table 4. Two major haplotypes, TCTACG CACG (frequency = 0.361) and TTTATATACA (frequency = 0.236), were predicted by M-fold. The results indicate the secondary structures of the two major haplotypes were different, which resulted in a change in free energy (Fig. 3).

3.5. Effect of haplotypes on abundance of mature miR-26a-5p transcripts

To analyze the effect of the two predominant haplotypes on mature miR-26a-5p, chicken follicle theca cells were transfected with

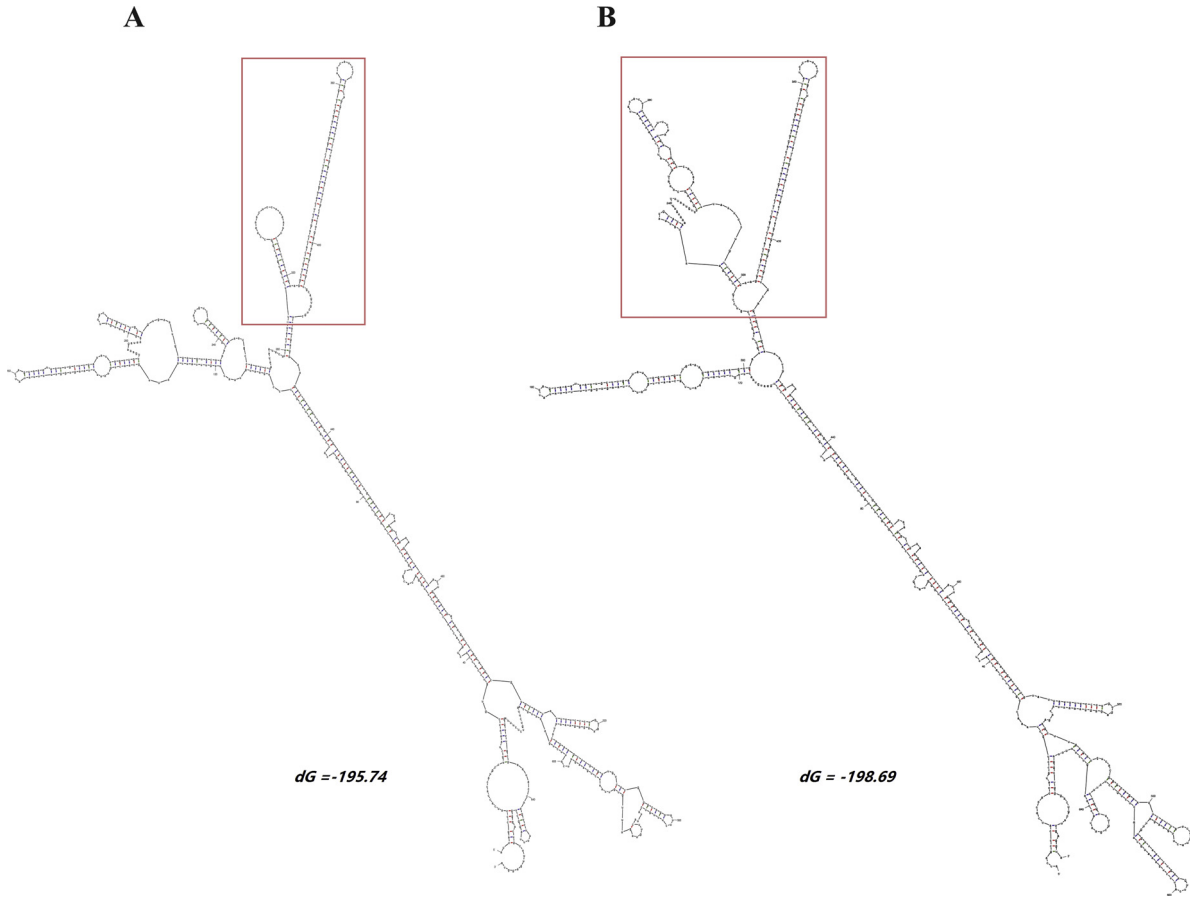

Fig. 3. Predicted secondary structure of the pri-miR-26a-5p with different haplotypes by M-fold software; (A) Secondary structure and free energy of haplotype TCTACGCACG; (B) Secondary structure and free energy of haplotype TTTATATACA.

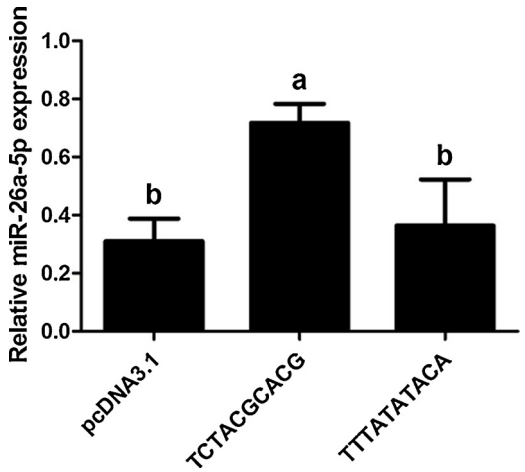

Fig. 4. Real-time PCR analysis of the effect of the two dominant haplotypes on abundance of mature miR-26a-5p; Transfected empty pcDNA3.1 vector was used as a control, and relative abundance of mature miR-26a-5p was normalized to 5S rRNA; Bar values depicted with different superscript letters are different $P < 0.05$.

a TCTACGCACG or TTTATATACA haplotype plasmid and both were detected using qPCR. The results indicate that the abundance of mature miR-26a transcripts in the cells with the TCTACGCACG haplotype was greater than that of cells with the TTTATATACA haplotype (Fig. 4).

4. Discussion

Increasing evidence suggests that SNPs in miRNA genes, including pri-miRNA, pre-miRNA and mature miRNA, may affect the function of miRNA, leading to phenotypic variation in animals. There has been identification of the first SNP in pre-miRNA, although this SNP had no contribution to microRNA processing (Iwai and Naraba, 2005). There has also be a report that the SNP at the eighth nucleotide (+ 8) of mature miR-125a inhibited the processing of pri-miRNA to pre-miRNA (Duan et al., 2007). Furthermore, one SNP in pre-miR-146a reduced the abundance of mature miRNA and enhanced the propensity for papillary thyroid carcinoma (Jazdzewski et al., 2008). Similarly, a SNP in miR-126-24 inhibited the processing of pri-miRNA to pre-miRNA, decreasing the abundance of mature miR-126 transcript (Harnprasopwat et al., 2010).

Results of studies indicate miR-26a was in large abundance in the ovaries of vertebrates (Ro et al., 2007; Zielak-Steciwko et al., 2014 Xu et al., 2016). In addition, miR-26a family members were reported to be involved in ovarian follicle cell proliferation and apoptosis (Liu et al., 2014; Kang et al., 2017). These previous findings indicate that miR-26a members have an important role in ovarian function. Little, however, is known about the SNPs in the miR-26a-5p gene. In the present study, there was identification of ten SNPs in pri-miR-26a-5p and analysis of the effects on values for chicken egg production variables. Statistical analysis indicated that the rs316365438 (C > T) of allele T had a positive effect on AFE and E32 in White Recessive Rock populations, and hens with the TT genotype had a greater total number of E32 and earlier AFE compared with hens of the other genotypes. Results of other studies also indicate there are SNPs in chicken miRNA genes. The SNPs (gga-miR1657 and gga-miR-1614-3p) located in the mature miRNA were associated with values for chicken production variables (Li et al., 2012, 2013). For the SNP (g. 5678784 A > T) at 95-bp upstream of gga-miR-1596, birds with the AA genotype of this SNP had less residual feed intake and a greater abundance of mature gga-miR-1596-3p miRNA transcript than birds with the AT or TT genotype (Luo et al., 2015). A SNP in pre-miR-1666 and pre-miR-1606 resulted in a change in the abundance of mature miRNA transcript and was associated with chicken carcass traits (Wang et al., 2015; Li et al., 2015); and a SNP in pre-miR-1658 was associated with an effect on growth and carcass traits at different developmental stages (Shi and Sun, 2017).

In the present study, the polymorphisms in the primary region of gga-miR-26a-5p altered the miRNA gene secondary structure and free energy. Results of previous studies indicated that a SNP in the miRNA stem was associated with lesser stability of the hairpin structure and a reduction in the product of mature miRNA. With a greater change in energy, the more likely it is that the product is affected, although there may be some exceptions and more experimental data are needed for validation (Sun et al., 2009; Gong et al., 2012). To further determine whether different haplotypes could lead to differences in abundances of mature miRNA transcript, chicken theca cells were transfected with two haplotype plasmids. Compared with the TTTATATACA haplotype, the abundance of mature miR-26a-5p of cells with the TCTACGCACG haplotype was greater. These data indicate that SNPs might be involved in changes if the processing efficiency of mature miRNA, affecting its function in translational processes.

In conclusion, in the present study there was identification of ten SNPs within chicken miR-26a-5p primary transcripts. The rs316365438 (C > T) SNP was associated with changes in values for chicken egg production variables. Different haplotypes, however, affected the abundances of mature miR-26a-5p. These results indicate there is a possibility that miR-26a-5p could have an important role in chicken ovarian function. Further characterization of these SNPs in pri-miR-26a-5p will be helpful in understanding miRNA biogenesis and the potential contribution of these polymorphisms to ovarian function in chickens.

Conflict of interest

The authors have no conflict of interest to declare about this manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anireprosci.2019.01.002.

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