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The Chicken *GGA-Mir-1658** Gene: Seed Region Polymorphisms, Frequency Distribution and Putative Targets

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Abstract: The aim of this study was to investigate the rs16681031 SNP located in seed regions of chicken *gga-mir-1658** gene with PCR-RFLP using PvuII nuclease in 6 chicken populations 180 individuals. The finding represents that genotype frequencies of the *gga-mir-1658** gene C>G has significant differences in Beijing Fatty and other chicken breeds. Bioinformatics analyses indicated that *gga-mir-1658** gene C>G polymorphism may alter target selection and secondary structure. The findings indicate that the rs16681031 SNP may exert profound biological effects in the formation of some special phenotype of chicken and enables functional annotation of *gga-mir-1658** gene.

Key words: *Gga-mir-1658**, chicken, SNP, breeding, annotation, China

INTRODUCTION

The rs16681031 SNP is located in seed regions of chicken *gga-mir-1658** gene which resides in the intron 13 of Guanine Monphosphate Synthetase (GMPS) gene (Griffiths-Jones *et al*., 2008). Many evidences indicated that microRNA-SNPs may modify various biological processes by influencing the processing and/or target selection of microRNAs having long ranging phenotypic effects (Mishra *et al*., 2008; Sun *et al*., 2009). This SNP has not been studied in details so far. Therefore, the objectives of the present study were to elucidate the effect of the rs16681031 SNP on *pre-gga-mir-1658*'s* secondary structure, investigate the distribution of the C>G polymorphisms of *gga-mir-1658* gene* among six chicken populations and identify putative target genes of *gga-mir-1658** gene.

MATERIALS AND METHODS

DNA samples: A total of 180 samples were obtained from 6 breeds including 30 Beijing Fatty chickens (BF), 30 Jiningbairi chickens (JB), 30 Langya chickens (LY), 30 Siyuwugu chickens (WG) (30), 30 Wenshangluhua chickens (WL) and 30 Leghorn chicken (LH). Genomic DNA was extracted from chicken venous blood through classical phenol-chloroform method.

PCR-RFLP: A 239-base pair (bp) fragment of the *gga-mir-1658** gene was amplified using forward (5'-ATAC CAGTGTGTGTTTCTCACA-3') and reverse (5'-GCCTCA CAGCAGGATTTACT-3') primers. The PCR reaction volume of 25 μL contained approximately 50 ng of genomic DNA, 1.25 mM Taq DNA polymerase, 2.5 μL of 1×PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP and 10 pM of each primer. Amplification conditions included an initial denaturation at 94°C for 4 min followed by 35 cycles at 94°C for 30 sec, 58°C for 30 sec and 72°C for 30 sec, followed by a final extension at 72°C for 10 min. The *gga-mir-1658** gene PCR product was digested was digested with 10 units of PvuII restriction enzyme and 10 μL of PCR product at 37°C overnight in a water bath. The digested products were detected by electrophoresis in 3.5% agarose gel stained with Ethidium Bromide (EB).

Secondary structure alterations of variant gga-mir-1658* precursors: The most stable secondary RNA structure with the lowest free energy for pre-gga-mir-1658* with G>C alleles were calculated using Mfold (Zuker, 2003). The absolute difference of free energy for pre-gga-mir-1658* with different alleles were used as the parameter for the assessment of the impact on secondary structure of pre-gga-mir-1658*.

Impact of SNP on *gga-mir-1658 target genes:** The chicken Unigene (NCBI) was scanned for potential gga-mir-1658* targets using the miRanda algorithm (version 3.1) (Enright *et al*., 2003) with the default parameters for score threshold (>130) and free energy threshold (<-16). The predicted targets were further filtered using more stringent criteria in which they must contain either:

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- A match between nucleotides 2-8 of the microRNA with the target sequence
- A match between nucleotides 2-8 of the microRNA with the target sequence (G:U base-pairing was not tolerated)

Statistical methods: The allelic frequencies were tested using the PopGene v.3.1 (Yeh *et al*., 1999). The heterogeneity between population samples was evaluated by Fisher's exact test. A $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

RFLP: The profile of *gga-mir-1658** gene G>C polymorphism is shown in gel photograph (Fig. 1). After digestion, the CC genotype had 176 bp and 61 bp bands, the GC genotype had 176, 61, 41 and 20 bp bands and the GG genotype had a 176, 41 and 20 bp bands.

Allele frequency, genetypic frequency: *gga-mir-1658** genotypes and alleles frequencies detected by PCR-RFLP in 6 chicken breeds were shown in Table 1. In 6 breeds most had 2 alleles A and G, 3 genotypes AA, AG and GG except WG chicken. The lowest frequency of the G allele was found in the WL (0.1833) and in the highest frequency in the BF (0.8000). In addition, the results of Fisher's exact test indicated that there was a significant difference for allele frequencies of G>C of the *gga-mir-1658** gene between the BF and other chicken breeds ($p<0.01$ or $p<0.05$).

Secondary structure alterations of variant gga-mir-1658* precursors: The G>C located in the seed region of gga-mir-1658* could introduce a base-pairing mismatch, alter free energy values create a new RNA bulge and alter the predicted RNA secondary structure with Mfold program (Fig. 2).

Impact of SNP on *gga-mir-1658 target genes:** The SNP is located in the crucial seed sequence of *gga-mir-1658** gene so it determines its complementarity to potential target genes affecting the functionality of both isoforms. Using the miRanda software, researchers predicted to have profoundly different target genes (82 genes for gga-mir-1658*-G and 67 genes for gga-mir-1658*-C) with only *PRPS2* and *VSX1* genes being shared by the two isoforms (Fig. 3 and Table 1). Of note, one of the shared genes both *PRPS2* and *GMPS* which *gga-mir-1658** hosted gene are involved in the purine metabolism processes (Kanehisa *et al*., 2010).

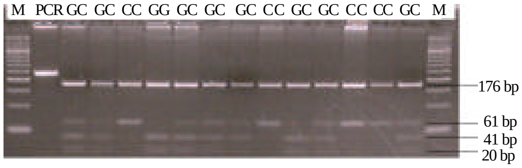

Fig. 1: PCR-RFLP patterns of the chicken gga-mir-1658*, M = Marker 50 bp ladder

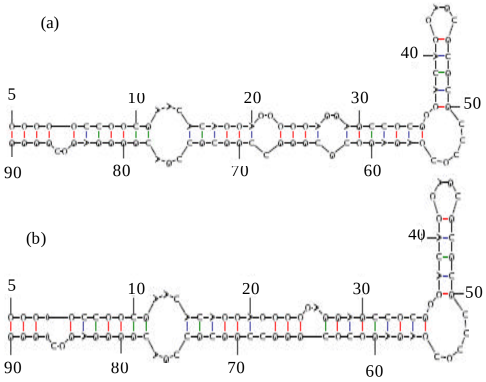

Fig. 2: The predicted structure of *pre-gga-mir-1658** (a) gga-mir-1658*-G, ΔG = -36.10 kcal moL^{-1} (b) gga-mir-1658*-C, ΔG = -40.80 kcal moL^{-1}

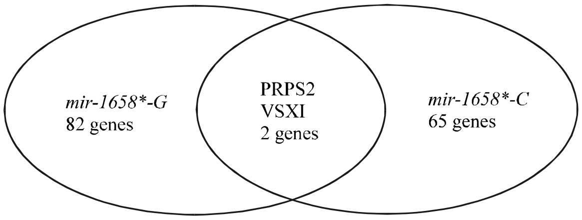

Fig. 3: Target genes of the mature products of *gga-mir-1658**

MicroRNAs comprise a growing class of non-coding RNAs that are believed to regulate gene expression via translational repression. Sequence variations within the *microRNA* genes could potentially influence the processing and/or target selection of microRNAs. Recently, Mir-146a-SNP (rs2910164) within the pre-miR-146a sequence reduced both the amount of pre-miR-146a and mature miR-146a and apparently affected the Drosha/DGCR8 processing step (Jazdzewski *et al*., 2008). In current study, researchers perform a systematic analysis of SNPs associated with *gga-mir-1658** gene. The results suggest that the gga-mir-1658* SNP have a larger impact on secondary structure and may alter the maturation of *gga-mir-1658**. Additionally, researchers also found the SNP would theoretically alter *gga-mir-*

Table 1: Allele frequencies, genetypic frequencies and the p value of Fisher's exact test of *gga-mir-1658** gene in 6 chicken breeds

gga-mir-1658	Genotypes			Allele frequency							
*G>C	CC	CG	GG	C	G	WL	WG	JB	LH	BF	LY
WL	19	11	2	0.8167	0.1833	-					
WG	13	14	0	0.6897	0.3103	0.1548	-				
JB	12	10	18	0.4138	0.5862	0.0113	0.3479	-			
LH	7	17	6	0.4833	0.5167	0.2186	0.1369	0.7142	-		
BF	1	10	19	0.2000	0.8000	<0.0100	<0.0500	<0.0100	<0.0100	-	
LY	7	15	8	0.4833	0.5167	<0.0100	0.0643	0.4648	0.8552	<0.01	-

Table 2: Impact of SNP on *gga-mir-1658** target genes

<table>
<tr><th>Targetgenes</th><th>gga-mir-1658*-G (kcal mol^{−1})</th><th>Targetgenes</th><th>gga-mir-1658*-C (kcal mol^{−1})</th></tr>
<tr><td>BARX1</td><td>3' G-G-TAG-TT-GTG-GTTGGGTGTCGAG 5'
| | :|| :| ||| |:|| ||||||| -30.78
5' CGCGGTCGGAGCACTCGGCGCACAGCTC 3'</td><td>DACH1</td><td>3' GGTA-GTTGTGGTTGGGTCTCGAG 5'
|| | :::| :||| ||||||| -25.38
5' CC-TGTGGC-TCAAGCCAGAGCTC 3'</td></tr>
<tr><td>NHLH1</td><td>3' GGTA-G-TTGTGGTTGGGTGTCGAG 5'
||:| | ::|||: ||||||||| -31.83
5' CCGTCCTGGCACT-GCCCACAGCTC 3'</td><td>DAN</td><td>3' GGTA-G-TTGTGGTT-G-G-GTCTCGAG 5'
|::| | :||| || | | ||||||| -21.80
5' CTGTCCTGACA-AAAGCTTCCAGAGCTT 3'</td></tr>
<tr><td>TXLNB</td><td>3' GG-TAGTTGTGGTTGGGTGTCGAG 5'
| ||||| || ||||||||| -26.62
5' ACAATCAA-ACAAACCCACAGCTA 3'</td><td>CBL</td><td>3' GGTAGTTG-TGGTT-GGGTCTCGAG 5'
:|| |||: | :| ||||||| -20.67
5' TCA-CAATGAGGGATCCCAGAGCTG 3'</td></tr>
<tr><td>MAFF</td><td>3' GGT-AGTTGTGGTT-GGGTGTCGAG 5'
|| || | | | ||||||||| -27.38
5' GCAGGGAA-A-C-ACCCCACAGCTC 3'</td><td>CDX1</td><td>3' GGTAGTT-GTGGT-TG-GGTCTCGAG 5'
|| || |||:| || ||||||| -19.78
5' AGAT-AAGCACTACACGGCAGAGCTT 3'</td></tr>
<tr><td>VSX2</td><td>3' GGTA-G-T-T-GTGGTTGGGTGTCGAG 5'
|||| | : : |||||||||||| -31.63
5' CCATGCCCCGTTCCCAGCTCACAGCTC 3'</td><td>POU1F1</td><td>3' GGTAGTTG-TGGTTG-GGTCTCGAG 5'
| |||: | |||| ||||||| -25.71
5' AAA-CAATGA-CAGTGCCAGAGCTC 3'</td></tr>
<tr><td>CCNA2</td><td>3' G-GTA-GTTGTGGTTGG-GTGTCGAG 5'
| | | | :|| |||| ||||||| -16.46
5' CTC-TGCTGCAGGAACTGCACAGCTG 3'</td><td>Cone-type transducin alpha subunit</td><td>3' GGTAG-T-TG-TGGTTG-G-GTCTCGAG 5'
||| | : :| || || | ||||||| -23.21
5' CCAGCTGTGCTCCCCACACACAGAGCTC 3'</td></tr>
<tr><td>MYOM2</td><td>3' G-GTAGTTGTGGTT-GGGTGTCGAG 5'
| :||: :|| ||| ||||||| -18.00
5' CTTATT-GCA-CAATTTCACAGCTG 3'</td><td>DIO3</td><td>3' GG-T-A-G-T-T-G-TGG-T-TGGGTCTCGAG 5'
:| | | | | | | :|| | | ||||||| -24.58
5' TCAAGTACTAGAACTGCCAATAGCCAGAGCTG 3'</td></tr>
<tr><td>IRF4</td><td>3'GG-T-AG-TTGT-GGTTGGGTGTCGAG 5'
| | |: :| | |||| ||||||| -18.85
5'GCAATTTGGAGATCCAAATCACAGCTG 3'</td><td>TUBB1</td><td>3' GG-TAGTTGTGGTTGG-GTCTCGAG 5'
|: :|: :||:: ||| ||||||| -17.84
5' CTGGTTTGCATT-ATTGCAGAGCTT 3'</td></tr>
<tr><td>FLNB</td><td>3'GGTAGTTGTGGT-TG-G-GTGTCGAG 5'
||:||::: |:: || | ||||||| -25.53
5'CCGTCGGT-CTGTACGTTCACAGCTC 3'</td><td>Cathepsin Z</td><td>3' GGTAGTTG-TGGT-T-GGGTCTCGAG 5'
:|| ||| :|:| | |||||||| -24.94
5' GTATAAACTGCTACAAGCCAGAGCTC 3'</td></tr>
<tr><td>HMGB1</td><td>3' GGTAGT-T-G-T-GGTTG-G-GTGTCGAG 5'
:||| | | | | |::|| ||||||| -20.21
5' TCAT-ATATCCAGCTGATAAACACAGCTT 3'</td><td>CRYM</td><td>3' GGTAGTTGT-G-G-T-TG-G-GTCTCGAG 5'
|| :||| | : : || | ||||||| -26.76
5' AAAT-GACATCATGGTACTCTCAGAGCTC 3'</td></tr>
<tr><td>EPT1</td><td>3' GG-TAGTTGT-GGTTGGGTGTCGAG 5'
|: | ||:|| | ||||||||| -27.82
5' CTGA-CAGCAGCAAATCCACAGCTT 3'</td><td>SUMO1</td><td>3' GGTAGT-TGT-GGT-T-GGGTCTCGAG 5'
|||| ::| ||| | |||||||| -23.59
5' AAATCATGTATCCATAACTCAGAGCTT 3'</td></tr>
<tr><td>Psmd3</td><td>3' GGTAGTTG-TGGTT-G-GGTGTCGAG 5'
|||| || ::: | | |||||||| -31.30
5' GCATC-ACTGTT-ACCCCCACAGCTC 3'</td><td>IL-17</td><td>3' GG-TAGTTG-TG-G-TT-GG-GTCTCGAG 5'
|| | ||:| |: || || ||||||| -20.78
5' CCTAACAGCGATGAAAATCCACAGAGCTG 3'</td></tr>
<tr><td>RNF185</td><td>3' GGT-AG-T-T-GTG-G-TT-GGGTGTCGAG 5'
||| |: : | ||| : :| | ||||||| -22.85
5' CCATTTGGAATCACGTCGAGTGCACAGCTT 3'</td><td>FADS2</td><td>3' GGTAGTTGTGGTTGGGTCTCGAG 5'
:: || || |::| ||||||| -21.46
5' TT-TC-AC-CTGA-GCAGAGCTC 3'</td></tr>
<tr><td>GRSF1</td><td>3' GGTA-GTTGTGGTT-GGGTGTCGAG 5'
|| :|: |:| | |||||||| -21.78
5' AAATATAGGATC-ATTTCACAGCTT 3'</td><td>GPSN2</td><td>3' GGTAGTTGTG-GTTGG-GTCTCGAG 5'
|| || :| : ||| |||||| -15.07
5' AAAT-AAGGCTTCATTGCAGAGCTG 3'</td></tr>
<tr><td>MOCO2</td><td>3' GGTAGTTGTGGT-TG-G-GTGTCGAG 5'
||: ::|:: || | ||||||| -20.22
5' GAATT-TTGCTGCACGTTCACAGCTC 3'</td><td>DPM1</td><td>3' G-G-TAGTT-GTG-GTTGGGTCTCGAG 5'
| : || || || ||| ||||||| -17.94
5' CTTAATAAAGAACACAA-ACAGAGCTA 3'</td></tr>
<tr><td>CALD1</td><td>3' GGT-AGT-T-G-T-GGTTG-GGTGTCGAG 5'
:|| ||| | : | |||| ||||||| -23.22
5' TCATTCATAATTAGCCAATATCACAGCTT 3'</td><td>PPIF</td><td>3' GG-T-AGTTGT-GGTTG-G-GTCTCGAG 5'
:: | | |:|| || || | ||||||| -23.95
5' TTGAGT-AGCAGCCTGCTTCCAGAGCTT 3'</td></tr>
<tr><td>CDH5</td><td>3' GGTAGTTGTGGTTGGGTGTCGAG 5'
|||| :| | :||||||||| -24.29
5' CCATGGA-A-AGATTCACAGCTC 3'</td><td>CLTB</td><td>3' GGTAGTTGT-G-GTTGG-GTCTCGAG 5'
:::|||| | | : ||| ||||||| -21.04
5' TTGTCAA-AGCTTTGCTGCAGAGCTC 3'</td></tr>
<tr><td>CD30</td><td>3' GGTAG-T-TGTGGTTGGGTGTCGAG 5'
|: || : | :|:||||||||||| -22.56
5' CT-TCTGGAGGCTAATTCACAGCTT 3'</td><td>Cell adhesion molecule 3</td><td>3' GGTAGTTGTGGTT-G-GGTCTCGAG 5'
||:|| || || | | ||||||| -25.95
5' CCGTC-ACTCC-ATCAGCAGAGCTT 3'</td></tr>
</table>

Table 2: Continue

Targetgenes	gga-mir-1658*-G (kcal mol^{-1})	Targetgenes	gga-mir-1658*-C (kcal mol^{-1})
RIKEN	3' GGT-AGTTGT-G-G-TTGGGTGTCGAG 5' |:| |:: || : | |||||||||| -28.98 5' CTATTTGCCATTCCAAATCCACAGCTC 3'	XRCC3	3' GGTAGT-TGTG-G-TTGGGTCTCGAG 5' || ||| | :| : | |||||||| -23.36 5' CC-TCAGAGGCTTTTA-TCAGAGCTC 3'
E-1 enzyme	3' GGTAGTTG-TG-GT-TGGGTGTCGAG 5' || |::|: :: || ||||||||| -23.99 5' CC-TTGATGGTGCACATCCACAGCTC 3'	CUEDC2	3' GGTAGT-TG-TGG-TTG-GGTCTCGAG 5' | ||| || :|| | |||||||| -25.50 5' GC-TCACACTGCCTTCCTCCAGAGCTC 3'
LEPROTL1	3' GGTAGTTGT-G--GT-TGGGTGTCGAG 5' ||||::||| : || || |||||||| -23.85 3' GG-T-AGTTG-TGGTTGG-GTGTCGAG 5'	MAB21L2	3' G-GTAG-TTG-TGGTTGGGTCTCGAG 5' |: | :|| : ||| ||||||| -23.51 3' GGTA-GTTG-TGGTTGGGTCTCGAG 5'
GTLF3B	3' GG-T-AGTTG-TGGTTGG-GTGTCGAG 5' || : || || ||| || ||||||| -26.95 5' CCTGCTC-ACTACCC-CCTCACAGCTC 3'	SBDS	3' GGTA-GTTG-TGGTTGGGTCTCGAG 5' ::|| ::|| |::|| ||||||| -19.99 5' TTATATGACAATTAA-ACAGAGCTC 3'
RIKEN	3' GG-TA-GTTGTGGTTG-GGTGTCGAG 5' || :| || |: ||| ||||||| -20.38 5' CCTGTAGAAAATGAGCTCCACAGCTG 3'	NCDN	3' GGTAGTTGT-G--G-T-TG-GGTCTCGAG 5' |||| |||| | | | | || ||||||| -31.34 5' CCAT-AACACCAGCAATACAGCAGAGCTC 3'
POLR3F	3' GGT-AGTTGTGGT-TG-GGTGTCGAG 5' | ||:|: :|| | ||||||| -19.20 5' AAACTCGAT-TCATTCTTCACAGCTA 3'	IPR328-like	3' GGTA-G-T-TGTGG-T-T-GGGTCTCGAG 5' || | | : ||:|| : | |||||||| -29.52 5' CC-TGCGGAACGCCTGGAGCCCAGAGCTG 3'
ANKRD1	3' GGTAGTTGTGGTTGGGTGTCGAG 5' |||||| :|| |||||||| -35.62 5' CCATCAA-GCCTTCCCACAGCTC 3'	VSX1	3' GGTAGTTGT-G-GTTG-GGTCTCGAG 5' |::| ||:| : :||| |||||||| -15.18 5' CTGTAAATAGTGTAATGTCAGAGCTT 3'
pDJA1	3' GGTA-GTTGT-G-GT-TGG-GTGTCGAG 5' :|| :|| | | | | ||| |||||| -16.59 5' ATATATAA-AGCAAATGTCACACAGCTG 3'	VSX1	3' GGT-A-G-TTGTGGTTGGGTCTCGAG 5' ||| | :| | || |||||||| -23.27 5' CCACCCCGGA-A-GAGGCCAGAGCTC 3'
TMIGD2	3' GGTAGT-TGTGG-TTGGGTGTCGAG 5' ||:| :|||| || ||||||| -28.38 5' AGATTAGGCACCGAA-ACACAGCTC 3'	FABP5	3' GG-T-AGTTGTGGTTG-G-GTCTCGAG 5' | | |||:: |||| ||||||| -24.32 5' ACAACTCAGT-CCAGTGGACAGAGCTC 3'
FNDC5	3' GGTAGTTG-T-G-G-T-TG--G-GTGTCGAG 5' ||| |||| : | | : || | ||||||| -25.11 5' CCAGCAACTGTCACTGGATGGCACACAGCTC 3'	NCBP2	3' GGTAGTTGTGG-T-TGG-GTCTCGAG 5' :::|: ||||: | ||| ||||||| -20.56 5' TTGTTTACACTAATATCACAGAGCTG 3'
OSBPL11	3' GGTAGTTGTG-G-TTGGGTGTCGAG 5' ||||| ||: ||||||||| -27.22 5' CCATCCTCATGAGAACTCACAGCTT 3'	ILK	3' GGTA-GTT-GT-G-G-TT-GGGTCTCGAG 5' || | ||| :: | : :| |||||||| -24.98 5' CC-TCCAAGTGCCTTTGACCCCAGAGCTA 3'
PKCb1	3' GG-TA-G-T-TG-TGGTTGGGTGTCGAG 5' |: :| : :| ||:||||||||| -26.13 5' CTGGTGGGGGGCAACTAGCCCACAGCTG 3'	SPIN1	3' GGTAGTTGTGGTT-GGGTCTCGAG 5' ||| | :| || ||||||||| -26.71 5' AGATC-A-GCGAATTTCAGAGCTC 3'
CTSA	3' GGTAGTT-GTGGTTGG-GTGTCGAG 5' || ||: |||| || ||||||| -27.79 5' ACACCAGTCACCTCCTGCACAGCTC 3'	LDHA	3' GGT-AGT-TGTG-GTTGGGTCTCGAG 5' || |:: |: | :|| |||||||| -20.64 5' ACACTTGTATTCTTAG-CCAGAGCTC 3'
MPP1	3' G-G-TAGTTGT-GGTTG-G--GTGTCGAG 5' | : ||: ||| |||| | ||||||| -24.43 5' CTTGATTTACACCCAGCTCAACACAGCTC 3'	FBXW11	3' GG-TAGT-TGT-GGT-T-G-G-GTCTCGAG 5' || | || |:| ||| | | | ||||||| -21.94 5' CCAAACACATACCCAGAATGTACAGAGCTT 3'
URM1	3' GGTAGT-T-G-T-G-G--T-T-GG-GTGTCGAG 5' |||||| | : | | | | | || ||||||| -27.73 5' CCATCACATTTACCCCCTATAACTGCACAGCTC 3'	SERF1A	3' GGTA-G-TTGTGGTT-G-G-GTCTCGAG 5' ||| | |: :|::| | | ||||||| -26.58 5' GCATCCCAGGGCTGACCGCGCAGAGCTC 3'
CMYA5	3' GG-TAGTTGTGGTTG-G-GTGTCGAG 5' |: || ||:||: || | ||||||| -23.50 5' CTGATGAATACT-ATGCACACAGCTC 3'	TRAPPC4	3' GGTAGTTGT-GGT-T-GGGTCTCGAG 5' || | ::|| |:: | ||||||||| -28.24 5' CC-T-GGCAGCTGTGTCCCAGAGCTC 3'
GALNS	3' GGTAG-TTGTGGTTGGGTGTCGAG 5' :| || :: : ||| |||||||| -20.85 5' TC-TCTGG-G-CAA-CCACAGCTC 3'	KLHDC2	3' GGTAGT-T-GT-GG-T-T-GG-GTCTCGAG 5' || || | || || : | || ||||||| -26.47 5' GCAGCAGAACAGCCTGTAGCCACAGAGCTC 3'
LOC772111	3' G-GTAGT-T-GTGGTTGGGTGTCGAG 5' | ::|:: | || || |||||| -16.23 5' CGTGTTGTATCAGGAAAGCACAGCTG 3'	KLHDC2	3' GG-TAGTTG-T-G-G-TTGG-GTCTCGAG 5' || :|||:| : | |||| ||||||| -26.59 5' CCTGTCAGCTGTCAGGAACTGCAGAGCTC 3'
PRPS2	3' GGTAGT-T-GTGGTTG-GGTGTCGAG 5' |: ||| : ::: || |||||||| -16.72 5' CT-TCAGGAGGTT-ACATCACAGCTT 3'	DSTN	3' GG-T-AGT-TGTGG-TTGGGTCTCGAG 5' :| | |:| :|||| || |||||| -21.34 5' TCAAGTTAGGCACCTAA-ACAGAGCTA 3'
ING4	3' GGTA-G-TTGTGGTTG-GGTGTCGAG 5' || | | |: :|| | |||||||| -26.18 5' CC-TGCCAGGGCCT-CGTCACAGCTC 3'	MCM3	3' GGTAGT-T-G-TGG-T-TGGGTCTCGAG 5' :|| || : : ||| : |||||||| -25.66 5' TCA-CAGGTTAACCTGCACCCAGAGCTG 3'
LOC770939	3' GG-T-AGTTGTG-GTTG-G--GTGTCGAG 5' || : || | | ::|| | ||||||| -17.11 5' CCTGTTC-CC-CTTGGCTCTGCACAGCTC 3'	BRPF3	3' G-GTAGT-TGTGGTTG-G-GTCTCGAG 5' | || || |::||:|| | ||||||| -21.22 5' CACA-CACATGCCGATGCACAGAGCTT 3'
XM_417742	3' GGTAGTT-GTGGT-T-GGGTGTCGAG 5' ||||| || | | | ||||||||| -26.44 5' TGATCAATCA-GAGAGCCCACAGCTT 3'	BRPF3	3' GGTAGTTG-T-GG-T-TG-GGTCTCGAG 5' |||| ::: | | |: | || ||||||| -20.75 5' CCAT-GGTGAGCTGATGCAGCAGAGCTC 3'

Table 2: Continue

<table>
<tr><th>Targetgenes</th><th>gga-mir-1658*-G (kcal mol^{-1})</th><th>Targetgenes</th><th>gga-mir-1658*-C (kcal mol^{-1})</th></tr>
<tr><td>XM_417742</td><td>3' GGTA-GTT-GT-G-GTTG-GGTGTCGAG 5'
|| ||| | | | | ||||||| -19.16
5' AAATGCAAGGAGCCCTCCTTCACAGCTT 3'</td><td>LOC770676</td><td>3' GGTA-G-TTGT-G-G-T-TGGGTCTCGAG 5'
|| | || | : | : | |||||| -20.19
5' AGATGCAAAGAGTGCTGGAGACAGAGCTC 3'</td></tr>
<tr><td>FAM18A</td><td>3' GGTA-GTTGTG-GTTGGGTGTCGAG 5'
|| || |: |||||||||| -22.52
5' TGATGGAA-ATACAGCTCACAGCTT 3'</td><td>GPA33</td><td>3' GGTAGT-TG-TG-G-TTG-GGTCTCGAG 5'
::|| : :| || | ||| |||||||| -20.55
5' TTATGGTGCTACGCAAACATCAGAGCTT 3'</td></tr>
<tr><td>MTMR3</td><td>3' G-GT-A-G-TTGTGGTTGG-GTGTCGAG 5'
| || | | :: : |:|| |||||| -22.95
5' CTCAGTGCTGG-G-CGACCTCACAGCTG 3'</td><td>RAB7</td><td>3' GGTAG-TTGT-G-GT--TG-GGTCTCGAG 5'
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5' GCATCTGAGAGCCCAGCACTTCAGAGCTC 3'</td></tr>
<tr><td>WDTC1</td><td>3'GGTAGTTGTG-GTTGGGTGTCGAG 5'
|| || ||| :||| |||||| -23.90
5' CCTTC-TCACGTAACACACAGCTC 3'</td><td>BVES</td><td>3' G-G-TAGT-TGTGGT-TGGGTCTCGAG 5'
| | |||| | ||:: ||||||||| -28.15
5' CGCGATCAGA-ACTGCACTCAGAGCTC 3'</td></tr>
<tr><td>RCC1 domain containing 1</td><td>3' GGTAGTTGTGGTTGGGTGTCGAG 5'
|: |:::: || | ||||||| -21.18
5' CT-TTGGT-CCTA-CCACAGCTC 3'</td><td>APCDD1L</td><td>3' GGTAGTTGTGGTTGGGTCTCGAG 5'
|::|||: ||: ||||||||| -25.84
5' CTGTCAG-ACT-GTCCAGAGCTC 3'</td></tr>
<tr><td>MAP3K14</td><td>3' GGTAGTTGTGGT-T-G-GGTGTCGAG 5'
||| |: | |:: | | ||||||| -27.93
5' CCA-CGTC-CTGCAGCCCCACAGCTC 3'</td><td>Clone 10h2</td><td>3' GGTAGTTGTG-G-TTGGGTCTCGAG 5'
|| | :|::: : || |||||||| -19.46
5' CC-T-GATGTGTGAA-CCAGAGCTC 3'</td></tr>
<tr><td>CYPOR</td><td>3' GGTAGTTG-TGGT-TGGGTGTCGAG 5'
||| | | | ||: ||||||||| -28.52
5' CCA-CCCCTCCCGTGCCCACAGCTC 3'</td><td>XM_422090</td><td>3' GGTAGTTGTGGT-TGG-GTCTCGAG 5'
|:| || :: | ||| ||||||| -22.38
5' CTA-AAA-GTGAGACCACAGAGCTC 3'</td></tr>
<tr><td>CYPOR</td><td>3' GGTAGTTGTGGTTG-GGTGTCGAG 5'
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5' GCATC-A-GCCAACAGCACAGCTT 3'</td><td>APBA2BP</td><td>3' GGTAG-TT-G-T-GGT-TGGGTCTCGAG 5'
||| | |: | : ||| ||||||||| -26.88
5' CCA-CGAGTCTGCCCATGTCCAGAGCTC 3'</td></tr>
<tr><td>FBXW5</td><td>3' GGT-A-GT-T-G-TGGTTGGGTGTCGAG 5'
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5' TCACTGCAGGGTCGCCTCCCCACAGCTT 3'</td><td>DNMT3B</td><td>3' GGT-AGTTGTGGTT-GGGTCTCGAG 5'
|| || || :::| ||||||| -25.65
5' ACATTCCAC-TTGAGCCCAGAGCTG 3'</td></tr>
<tr><td>OCLN</td><td>3' G-G-T-AGT-TGT-GGT-TGGGTGTCGAG 5'
| : | || | | :|| | ||||||| -23.18
5' CTTGAGACAGAAAGTCAGAAACACAGCTC 3'</td><td>GTLF3B</td><td>3' GGTAGTTGTGGT-T-G-G-GTCTCGAG 5'
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5' CCATT-GCAGCAGAGCACGCAGAGCTT 3'</td></tr>
<tr><td>RNF166</td><td>3' GGT-A-GTTG-TGGTT-GGGTGTCGAG 5'
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5' TCAGAGC-ACGGCCAGCCCCACAGCTC 3'</td><td>XM_429703</td><td>3' GGTAGTT-GT-GGT-T-GG-GTCTCGAG 5'
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5' TTAT-AAGGGTCTATGGCCACAGAGCTG 3'</td></tr>
<tr><td>LDLR</td><td>3' GGTAGT-TGTG-GTTGGGTGTCGAG 5'
|| || |::| ||| ||||||| -18.96
5' ACA-CAGATGWGCAAACCACAGCTW 3'</td><td>KLHDC3</td><td>3' GGT-AGTTGTGGT--TG-G-GTCTCGAG 5'
|: |||| |||| || | ||||||| -25.28
5' GCGTTCAA-ACCAGGATGCTCAGAGCTT 3'</td></tr>
<tr><td>CTSO</td><td>3' GGTAG-TTG-T-GGTTG-GGTGTCGAG 5'
||||| ||: | ::|| |||||| -21.46
5' CCATCTAATAAGATGACAACACAGCTT 3'</td><td>KRTCAP3</td><td>3' G-G-TA-G-TTGTG-GTT-GG-GTCTCGAG 5'
| : :| | ::::| | | | || |||||| -20.18
5' CTTGGTGCTGGTGCTCCACCCGCAGAGCTG 3'</td></tr>
<tr><td>CTSO</td><td>3' GGTAGTTG-TG-GTTG-GGTGTCGAG 5'
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5' CC-TC-TCTTCTCTACTTCACAGCTA 3'</td><td>KRTCAP3</td><td>3' GGTAGTT-GTGGTTG-G-GTCTCGAG 5'
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5' GTGTCAGTCTCTTACATGCAGAGCTG 3'</td></tr>
<tr><td>Occludin</td><td>3' GGT-A-GTTGT-GG-TT-GGGTGTCGAG 5'
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5' CCAGAATGGTACCCTGAGCACACAGCTC 3'</td><td>Clone1n18</td><td>3' GGT-A-G-TTGTGGTTGG-GTCTCGAG 5'
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5' AAATTGCCAACACTTACCTCAGAGCTC 3'</td></tr>
<tr><td>NM_205099</td><td>3' GGTAGTTGTGGTTGGGTGTCGAG 5'
:| ||:| ||| ||||||| -23.08
5' AGGTGAATA-CAG-CCACAGCTC 3'</td><td>CHMP6</td><td>3' GGTAGTTGTGGTT-GGGTCTCGAG 5'
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5' CCG-CAGCACTGAGTGCAGAGCTG 3'</td></tr>
<tr><td>FLOT2</td><td>3' GG-T-A-GT-TGTGGT-TG-GGTGTCGAG 5'
|| | | :: :||:|| || |||||||| -27.03
5' CCGACTTTGTGCATCAGGTGCCACAGCTC 3'</td><td>HYPE</td><td>3' GGTA-GTT-GT-GG-T-TG-GGTCTCGAG 5'
:|| || :| :| : || |||||||| -20.54
5' GTATAAAAGTATTCTGCACTTCAGAGCTC 3'</td></tr>
<tr><td>AANAT</td><td>3' GGTA-G-TTGTGGTTGGGTGTCGAG 5'
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5' CC-TGCTGGCCCCAG-CCACAGCTC 3'</td><td>Clone23i24</td><td>3' G-GTAGTTGTGGTTGGGTCTCGAG 5'
| ::|||::::: | |||||||| -23.89
CTTGTCAGTGTT-G-CCAGAGCTC 3'</td></tr>
<tr><td>CSRP1</td><td>3' GGTAGTTGTGGTTGGGTGTCGAG 5'
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5' CC-CCGGCACGGGCACACAGCTC 3'</td><td>GZMA</td><td>3' GGTAGTTG-T-G-GTT-GG-GTCTCGAG 5'
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5' GAAT-AGCTTTTGCAGTTTGCAGAGCTA 3'</td></tr>
<tr><td>CNTF</td><td>3' G-GTAGTTGTGGTTGG-GTGTCGAG 5'
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5' CTC-TC-G-GCT-CCTGCACAGCTC 3'</td><td>Keratin</td><td>3' GGTAGTTG-TG-G-TT-G-GGTCTCGAG 5'
|| |||:| : | :| | |||||||| -27.04
5' CCTTCAGCTTTGCTGAGCTCCAGAGCTC 3'</td></tr>
<tr><td>NAT</td><td>3' GGTA-GTTGTGGTTG-GGTGTCGAG 5'
|||| :||: :| || |||||| -18.68
5' CCATATAAT-TC-ACAACACAGCTA 3'</td><td>TRAPPC2</td><td>3' GGTA-G-TTGT-GGTT-G-GGTCTCGAG 5'
|| | || | |: | | |||||| -18.76
5' AAATACAAAGATCTTATCAGCAGAGCTT 3'</td></tr>
<tr><td>CRYM</td><td>3' GG-TAGTTGTGG-TTGGGTGTCGAG 5'
| ||| |||| :| |||||| -20.92
5' GCAATC-CCACCTGA-ACACAGCTT 3'</td><td>CHMP2A</td><td>3' GGTAG-TTGTGGT-TGG-GTCTCGAG 5'
:| || :||| || ||| |||||| -23.27
5' TC-TCTGACAACACACTACAGAGCTT 3'</td></tr>
<tr><td>CDKN1A</td><td>3' GGTAGTTG-T-G-G-TTGGGTGTCGAG 5'</td><td>DM64</td><td>3' GGT-A-GT-TGTGGTTG-G-GTCTCGAG 5'</td></tr>
</table>

Table 2: Continue

<table>
<thead>
<tr><th>Targetgenes</th><th>gga-mir-1658*-G (kcal mol^{-1})</th><th>Targetgenes</th><th>gga-mir-1658*-C (kcal mol^{-1})</th></tr>
</thead>
<tbody>
<tr><td></td><td>|| || || : : : :| ||||||| -16.64
5' CC-TC-ACTGGTGTGGA-TCACAGCTG 3'</td><td></td><td>:|| | |: :| |||| | ||||||| -26.04
5' TCAGTACGTGC-CCAGCACACAGAGCTC 3'</td></tr>
<tr><td>CAT-catalase</td><td>3' GGTAG-TTGTG-GTTGGGTGTCGAG 5'
|:||| : ::| :|| ||||||| -19.23
5' CTATCTGTTGCTTAAACCACAGCTG 3'</td><td>PRKAB1</td><td>3' GG-TAGTTGT-GGTTGGGTCTCGAG 5'
|: :|||| : |||| ||||||| -24.35
5' CTGGTCAA-GTCCAG-CCAGAGCTG 3'</td></tr>
<tr><td>RER1</td><td>3' GGT-A-G-TTGTGGTTG-G-GTGTCGAG 5'
||| :| | ||| | ||||||| -20.16
5' GCAGTAGGGAGAAAAACACACACAGCTC 3'</td><td>C1orf130</td><td>3' GGTAGT-TGTGGTT-G-G-GTCTCGAG 5'
||| |: |||::| | | ||||||| -22.23
5' CCA-CGCACACTGATTTTGCAGAGCTT 3'</td></tr>
<tr><td>CANX</td><td>3' GG-TAGTTGTG-GTTGG-GTGTCGAG 5'
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5' CCTTTC-TC-TGTGACTACACAGCTT 3'</td><td>PEMT</td><td>3' GGTAGTTGTGGTTGG-GTCTCGAG 5'
|: || |||| ||| ||||||| -26.60
5' CTTTC-CCACC-ACCGCAGAGCTC 3'</td></tr>
<tr><td>BCL2L13</td><td>3' GGTAGT-TGT-G-GT-TGGGTGTCGAG 5'
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5' CCTTCAGATAGCGGAGATGCACAGCTT 3'</td><td>DZIP1</td><td>3' GG-TAGTTGTGGTT-GG-GTCTCGAG 5'
| || ||:||: | || ||||||| -21.70
5' ACAATGAATACT-GTTTACAGAGCTC 3'</td></tr>
<tr><td>PLEKHB1</td><td>3' GGTAGTTGTGGTTGGGTGTCGAG 5'
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5' CCATC-CCACCCACCCACAGCTT 3'</td><td>PRPS2</td><td>3' GG-TAGT-TGTG-G-T-T-GGGTCTCGAG 5'
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5' TTGGT-ATACTTGTAAAATTCCAGAGCTT 3'</td></tr>
<tr><td>NRIP3</td><td>3' GGTA-GTTGT-G-GT-TG-GGTGTCGAG 5'
||| ||::| : :| || ||||||| -21.85
5' ACATACAGTATTTTATATAACACAGCTC 3'</td><td>ANKRD13C</td><td>3' GGTA-G-TTGTGGTTGG-GTCTCGAG 5'
::|| | |: | : ||| ||||||| -22.95
5' TTATCCCAG-AGT-ACCACAGAGCTT 3'</td></tr>
<tr><td>TRAF4</td><td>3' GGTAGTTGT-GGT-TG-G-GTGTCGAG 5'
|| ||: | :|| || | ||||||| -24.43
5' GCA-CAGGAGTCAGGTGCTCACAGCTC 3'</td><td>RASL11B</td><td>3' GGT-AG-T-TG-T-GGTTGGGTCTCGAG 5'
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5' CCAGACTGAATGATCTAACACAGAGCTT 3'</td></tr>
<tr><td>LEPROT</td><td>3' GGTAGTTGTGGTTGGGTGTCGAG 5'
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5' TCA-CAATA-CAGCATACAGCTC 3'</td><td>AJ720340</td><td>3' GGTAGTTG-T--G-G-T-T-G-G-GTCTCGAG 5'
|||| |:| : | | : | | | ||||||| -25.75
5' CCATGAGCTGCTCCCTGTGCCCCTCAGAGCTC 3'</td></tr>
<tr><td>RAMP1</td><td>3' GG-T-AGTTGTGGT-T-G-GGTGTCGAG 5'
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5' CTGGGT-AATGGGAGAGTGACACAGCTC 3'</td><td></td><td></td></tr>
<tr><td>KLHDC8B</td><td>3' GGTA-GT-TG-T-G-GT-T-G-G-GTGTCGAG 5'
|| | | || : || | | | ||||||| -23.66
5' CC-TGAAGACGGGGACAGGGTGCGCACAGCTC 3'</td><td></td><td></td></tr>
<tr><td>TMEM111</td><td>3' GG-T-AGTTGTGGTT-GGGTGTCGAG 5'
|| | :::|| ::| |||||||| -22.71
5' CCAAGGTGGCAGTGACCCCACAGCTG 3'</td><td></td><td></td></tr>
<tr><td>FZD8</td><td>3' GGTAG-TTG-T-GG--T--TGGGTGTCGAG 5'
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5' CCA-CTAATGGTCCTTATTAGCCACAGCTT 3'</td><td></td><td></td></tr>
<tr><td>CHRNA7</td><td>3' GGTAG-TTGTGG-TTGGGTGTCGAG 5'
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<tr><td>LDLRAP1</td><td>3' GGTAG-TT-G-T-GGTTGG-GTGTCGAG 5'
|| | || : | :| || ||||||| -22.06</td><td></td><td></td></tr>
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5' CCAAGTCAGATGAAGTTACAGCACAGCTT 3'</td><td></td><td></td></tr>
<tr><td>K123</td><td>3' GGTAGTTGTGGTTG-GGTGTCGAG 5'
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5' GCAT-GGGAGGAACATCACAGCTC 3'</td><td></td><td></td></tr>
<tr><td>HLF</td><td>3' GGT-AGTTGTGGTTGGGTGTCGAG 5'
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5' TCAGTTGGTATC-A-TCACAGCTC 3'</td><td></td><td></td></tr>
<tr><td>GPC4</td><td>3' GGT-AGTTG-TGGT-T-GG-GTGTCGAG 5'
||| || :| : || | | || |||||| -24.01
5' CCACTC-GCTGGCACATCCTCACAGCTG 3'</td><td></td><td></td></tr>
<tr><td>RAB18</td><td>3' GGTAG-T-TG-TGGT-TGGGTGTCGAG 5'
:|||: | :| :|:: | |||||||| -25.59
5' TCATTAATGCTGCTGTA-CCACAGCTC 3'</td><td></td><td></td></tr>
<tr><td>SNUPN</td><td>3' GGT-AGTTGTGGTT-GGGTGTCGAG 5'
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5' CCGCTCCGC-CTGAGCCCACAGCTT 3'</td><td></td><td></td></tr>
<tr><td>HIVEP2</td><td>3' GGTAGTTGTG-GTTGG-GTGTCGAG 5'
|:| | | | | | || ||||||| -21.38
5' GCGT-A-C-CTCT-CCGCACAGCTT 3'</td><td></td><td></td></tr>
<tr><td>NCBP1</td><td>3' GGTA-GTTGTGGTTGGGTGTCGAG 5'
:| | | ||| |||| ||||||| -25.01
5' TC-TGCTACAGCAACACACAGCTC 3'</td><td></td><td></td></tr>
</tbody>
</table>

Table 2: Continue

Targetgenes	gga-mir-1658*-G (kcal mol^{-1})	Targetgenes	gga-mir-1658*-C (kcal mol^{-1})
CNOT1	3' GGTAGTTG-TGGT-TG-G-GTGTCGAG 5' ||||||| ||| || | | |||||||| -32.55 5' CCATCAACTTCCAGACGCTCACAGCTT 3'		
VSX1	3' GGTAGT-TGTGGT-T-GGGTGTCGAG 5' ||: | | :||| | ||||||||| -26.10 5' CCGGAAGAGGCCAGAGCTCACAGCTT 3'		
TNFRSF1A	3' GGTAGT-T-GTGGT-TGGGTGTCGAG 5' :::| | | | | || ||||||||| -21.81 5' TTGTGAGAGGA-CATGCCCACAGCTG 3'		

*1658*s* targets selection and >145 different mRNAs were predicted. From the result of genetic polymorphism at *gga-mir-1658** were found to differ significantly in 6 typical chicken breeds. This result has provided instructive significance for study the phenotypic differences between BF chickens and other populations.

CONCLUSION

Findings presented in this study indicated that the rs16681031 SNP in seed regions of chicken *gga-mir-1658** gene may be a functional sites which plays an important roles in the formation of some special phenotype of BF chicken. Furthermore, the bioinformatics analysis also provides a basis for functional annotation of *gga-mir-1658** gene orthologs in other species.

ACKNOWLEDGEMENTS

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