



Prolactin gene association with chicken egg production traits

JING FAN¹, LIN-LIN JIANG², XIAO-HUI LIU³, WEI-FENG ZHANG⁴, BIN WANG⁵, YU-XIANG SHI⁶ and
TENG-HE MA⁷

Hebei University of Engineering, Handan 056 021 China

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Prolactin (PRL) is a peptide hormone which is synthesized and secreted by the specialized cells in vertebrate anterior pituitary and regulates egg production in birds (Li *et al.* 2013, Xu *et al.* 2015). Higher circulating PRL levels are accompanied by an increase in incubation behaviour. Furthermore, PRL was found to promote ovarian follicle growth as well as egg-laying performance by enhancing the expression of LH receptor (LHR) on gonadal cells (Reddy *et al.* 2007).

Taihang chicken is a Chinese domestic breed, which is distributed in Hebei province among the Taihang Mountain in China, combined with good quality of egg and meat, green skin and jute feathers. Taihang chicken was named in 2016. It has the characteristics of light-body type, resistance of forage, strong resistance and adaptability. However, Taihang chicken has relatively low egg production. Average egg number at 500 days of age (EN 500) is 160–180. In this study, PRL was taken as candidate gene to identify the single nucleotide polymorphisms (SNPs) and their association with egg production to improve the egg production performance in the Taihang chicken.

Taihang chicken in Hebei Tiankai Poultry Technology Co. Ltd. (Hebei, China) were randomly selected to collect the blood samples and egg production data. Each bird was kept in a cell and fed a commercial diet and had free access to feed and water. The temperature of house was between 20 and 28°C in the whole year, with a photoperiod of 16 h light/day at 15 lx. The traits, viz. age at first egg (AFE), total egg number at 300 days (EN 300) and 500 days of age (EN 500) were observed. All experimental procedures involving animals were performed according to authorization granted by the Chinese Ministry of Agriculture.

Genomic DNA of all samples was extracted from whole blood using the Universal Genomic DNA Kit (Kangwei, China). Three pairs of primer were designed to amplify PRL (Gene Bank Accession no. NC_006089.4) by Primer

5 (Table 1). Polymerase chain reaction (PCR) was carried out in 20 µl reaction volume, which contained 50 ng of genomic DNA, 0.5 µl each primer (10 pmol), 10 µl 2× Es Taq MasterMix (Kangwei, China) and deionized water. The amplification protocol comprised of an initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at specified temperature (Table 1) for 30 sec, extension at 72°C for 1 min and then a final extension at 72°C for 7 min. All the PCR products were purified by DNA Clean-up Kit (Kangwei, China) and sequenced by ABI prism 3100 genetic analyzer (Songon, China). The sequences were aligned by DNASTAR software.

Allelic and genotypic frequencies were calculated using EXCEL. Association analysis of SNPs with egg production were determined by ANOVA using general linear model by SPSS 14.0 software.

SNP as genetic marker could be used to select animal to improve efficiency. Recently, Many studies examined correlations between markers of candidate genes and chicken egg production (Han *et al.* 2014, Wolc *et al.* 2014). The 5'-UTR of PRL gene was polymorphic, and bunch of studies had associated it with egg production in chicken (Bhattacharya *et al.* 2011a), but limited research focus on the CDS and UTR. In present study, six SNPs of PRL gene were found by direct sequencing method in Taihang chicken. Among them, three SNPs were located in CDS region and three in 3'-UTR (Fig. 1). Three SNPs, viz. g.4603, g.8823 and g.8885 were selected to determine the genotype and allele frequencies, and also associated with the egg traits. The distribution of genotypes and allele frequencies is given in Table 2. For SNPs g.4603, allele C was dominant as compared to allele T, but for g.8823 and g.8885, allele T and G were dominant than allele C. In SNPs g.4603, genotype CT was dominant as compared to CC and TT, but for g.8823 and g.8885, genotype TT and GG were more dominant as compared to CC and CT/CG, as also reported by Li *et al.* (2013). Genotypes did not differ from the expected Hardy-Weinberg equilibrium ($P > 0.05$). The g.4603 site was associated with EN 300 ($P < 0.05$), while the g.8823 was significantly associated with EN 300 ($P < 0.01$) (Table 3). In the previous study, T8052C and G8113C in exon 5 of PRL gene (Gene Bank Accession no.

Present address: ^{1,2,3,4,5,6}Scientist (1196868353@qq.com, 2960188030@qq.com, 812055317@qq.com, 113381@qq.com, hdwangbin@126.com, 763072035@qq.com), ⁷Principal Scientist (matenghe@126.com), Division of Animal Science.

Table 1. Primers sequence used for screening PRL gene polymorphisms

Primer	Sequence(5'–3')	Gene region	Product size (bp)	T _m (°C)
PF1	ACATCGGGTACTCTGAGCCAT	chr2:58774410-58775291	882	60
PR1	TCTTCCCCACACTCTATCTC			
PF2	CTTAAACCAATCTTCACCT	chr2:58778089-58779007	919	53
PR2	ATGCCTGAAAGTTACTGATG			
PF3	TTTTGAAGTGCCGCCTAAT	chr2:58778841-58779203	363	55
PR3	CATGGATACCACTTGTGT			

Table 2. Genotype and allele frequencies of different SNPs in Taihang chickens

Number	SNP	Genotype frequency			Allele frequency		χ^2
		CC	CT/CG	TT/GG	C	T/G	
151	g.4603	0.3311	0.6556	0.0133	0.6590	0.3410	1.76
	g.8823	0.1391	0.2583	0.6026	0.2682	0.7318	3.67
	g.8885	0.1722	0.2649	0.5629	0.3046	0.6954	1.20

$\chi^2_{0.05(2)}=5.99$; $\chi^2_{0.01(2)}=9.21$.

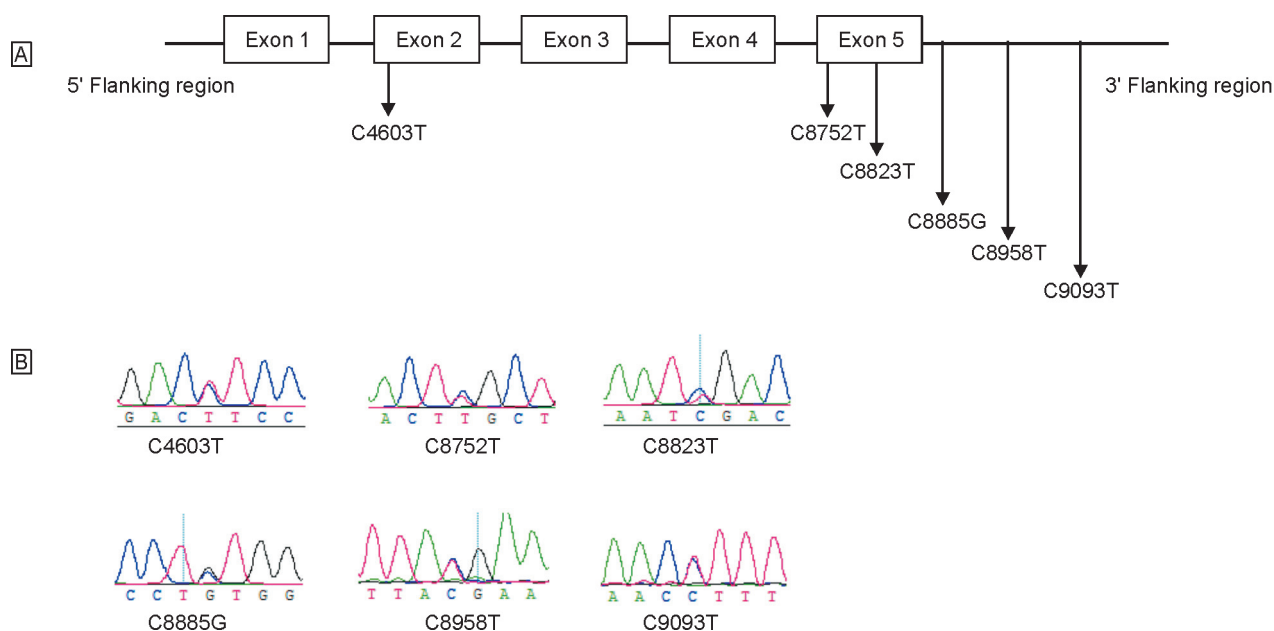
Fig. 1. SNPs analysis of the PRL gene. **A.** SNPs site in PRL gene, **B.** Sequencing alignment SNPs in PRL gene.

Table 3. Association of PRL genotypes with egg production traits in Taihang chickens

SNP	Trait	Genotype		
		CC	CT/CG	TT/GG
g.4603	AFE	172.58±15.11	173.74±14.72	165.00±14.14
	EN 300	180.48±13.69 ^a	171.64±13.35 ^b	172.26±15.28 ^b
	EN 500	174.46±14.33	172.28±15.57	173.33±14.69
g.8823	AFE	92.72±16.00	94.40±12.64	104.50±3.54
	EN 300	83.71±8.88 ^A	96.10±14.36 ^B	98.19±13.22 ^B
	EN 500	95.88±18.37	95.90±14.91	95.44±11.76
g.8885	AFE	193.98±30.41	188.49±29.04	188.00±25.46
	EN 300	181.24±29.16	193.90±22.65	190.85±31.78
	EN 500	188.08±31.91	195.23±28.16	188.66±29.26

Different superscript denote statistically significant differences between genotypes (^{A,B}P<0.01; ^{a,b}P<0.05).

AF288715) were associated with chicken egg production (Li *et al.* 2013), though these two SNPs were identical with our g.8823 and g.8885, but we did not observe any linkage in these 2 SNPs.

In conclusion, SNPs were selected with whole gene of PRL and associated with egg traits in Taihang chicken. The SNP g.4603 and g.8823 were significantly associated with EN 300. Hence, these two SNPs in PRL gene could be used as the potential molecular markers for egg production traits in chicken.

SUMMARY

In this study, using the direct sequencing technology, 6 SNPs were identified in PRL gene, among which, 3 SNPs were present in coding region and 3 in 3'UTR. Mutations in coding region did not change the protein sequences.

SNPs g.4603, g.8823 and g.8885 were utilized for association test with AFE, EN 300 and EN 500. SNP g.4603 was associated with EN 300, whereas the SNP g.8823 was significantly associated with EN 300. These two SNPs in PRL gene could be used as the potential molecular markers for egg production traits selection in Taihang chicken.

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REFERENCES

- Bhattacharya T K, Chatterjee R N, Sharma R P, Niranjana M and Rajkumar U. 2011. Associations between novel polymorphisms at the 5'-UTR region of the prolactin gene and egg production and quality in chickens. *Theriogenology* **75** (4): 655–61.
- Han C, An G and Du X. 2014. Three novel single nucleotide polymorphisms of the 3-hydroxy-3-methylglutaryl coenzyme A reductase gene associated with egg-production in chicken. *Folia Biologica* **62**(3): 203–09.
- Li H F, Shu J T, Du Y F, Shan Y J, Chen K W, Zhang X Y, Han W and Xu W J. 2013. Analysis of the genetic effects of prolactin gene polymorphisms on chicken egg production. *Molecular Biology Reports* **40**(1): 289–94.
- Reddy I J, David C G and Raju S S. 2007. Effect of suppression of plasma prolactin on luteinizing hormone concentration, intersequence pause days and egg production in domestic hen. *Domestic Animal Endocrinology* **33**(2): 167–75.
- Wolc A, Arango J, Jankowski T, Dunn I, Settar P, Fulton J E, O'Sullivan N P, Preisinger R, Fernando R L, Garrick D J and Dekkers J C. 2014. Genome-wide association study for egg production and quality in layer chickens. *Journal of Animal Breeding and Genetics* **131**(3): 173–82.
- Xu Z Q, He J, Ji C L, Zhang Y, Nie Q H, Zhang D X and Zhang X Q. 2015. Polymorphisms in the 5'-flanking regions of the GH, PRL, and Pit-1 genes with Muscovy duck egg production. *Journal of Animal Science* **93**(1): 28–34.