Association of Polymorphisms for Prolactin and Prolactin Receptor Genes with Broody Traits in Chickens

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ABSTRACT Prolactin (PRL) is generally accepted as crucial to the onset and maintenance of broodiness in avian species. The prolactin receptor (PRLR) plays an important role in the PRL signal transduction cascade. Two candidate genes, *PRL* and *PRLR*, were screened for polymorphisms in the chicken, and their genetic effects on broodiness were evaluated. Pedigreed hens (n = 155) of the Blue-shell chicken, a Chinese local breed, were observed for phenotypic broody traits including nesting days, broody days, repeats of broody cycles, and duration of broodiness. For polymorphism analysis, White Leghorns, Hy-Line brown egg layers, Avian broilers, and some other Chinese local breeds were included. Fifteen sets of primers were used to amplify the nucleotide sequences of the promotor of *PRL* and exons of *PRLR*. The PCR products were screened for polymorphisms using single-stranded conformational polymorphism protocol. Sequencing revealed a 24-bp insertion occurring in the promotor, −377 ~ −354, of *PRL* (GenBank accession no. AB011434). A single nucleotide polymorphism (SNP), A9026G (GenBank accession no. AY237377), in exon 3 of *PRLR* was also detected, which led to a nucleotide transition in the 5′-untranslated region (5′-UTR) of *PRLR* cDNA. Two SNP, T14771C and G14820A (GenBank accession no. AY237376), were detected in exon 6 of the *PRLR*. The T14771C transition led to an amino acid variation, Leu340Ser, in PRLR, whereas the G14820A transition was a synonymous mutation. An association analysis showed that the genetic polymorphisms at *PRLR3* and *PRLR6* were not related to broodiness ($P > 0.05$), whereas the individuals without the insertion sequence at *PRLpro2* were associated with broody traits ($P < 0.05$) and the incidence (>30%) of typical broody of genotypes +/− and −/− was higher ($P < 0.01$) than that of +/+. In addition, all White Leghorns were +/+ for *PRLpro2*, whereas local breeds with very strong broodiness were nearly all −/−. Homozygous insertion of the 24-bp sequence in the *PRL* promoter may decrease the expression of PRL, leading to nonbroodiness. The results suggested that *PRLpro2* could be a genetic marker in breeding against broodiness in chickens.

(*Key words*: broodiness, chicken, prolactin gene, prolactin receptor gene, single nucleotide polymorphism)

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INTRODUCTION

Broodiness is observed in most breeds of domestic fowl with the exception of the White Leghorn, which has undergone long-term artificial selection to minimize phenotypic expression of this behavior. Broodiness basically means a hen sits on her eggs for the purpose of hatching the embryos. Broodiness in chickens is usually associated with increased body temperature, reduced feed and water intake, frequent nest occupancy, turning and retrieval of eggs, aggressiveness or defensive behaviors, and characteristic clucking (Romanov et al., 2002). Hens cease egg production during the incubation period. Due to the adoption of artificial incubation technology, broodiness is no longer required in modern poultry production.

The inheritance of broodiness is complex with a long history of investigations on the subject. Although occurrences of broodiness can be effectively reduced by artificial selection, eradication is a slow process (Hays and Sanborn, 1939), suggesting polygenic inheritance for the condition that may also be viewed as a threshold trait. There are several genetic explanations for the behavior. Some researchers proposed autosomal inheritance (Goodale et al., 1920; Hays, 1940), whereas other researchers reported that genes on the sex chromosome contribute to broodiness (Saeki and Inoue. 1979). More recent studies have demonstrated that incubation behavior is not controlled by major genes on the Z chromosome and pro-

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Abbreviation Key: PRL = prolactin; PRLR = prolactin receptor; SNP = single nucleotide polymorphism.

posed that at least 2 dominant autosomal genes are involved, one causing and the other inhibiting the behavior with equal influence (Romanov et al., 1999, 2002)

Physiologically, it has been well established that prolactin (PRL) plays an important role in the onset of incubation of hens (Sharp et al., 1988; March et al., 1994; Ohkubo et al., 1998). Increased plasma PRL concentration is associated with the occurrence of broodiness (Burke and Dennison, 1980; Bacon et al., 1983). During incubation, PRL mRNA reaches its highest level (Talbot et al., 1991; Karatzas et al., 1997), which infers that PRL is important in the maintenance of broodiness. A turkey that is injected with PRL when in egg production will become broody (Youngren et al., 1991). Nest deprivation by broody female turkeys results in a significant decrease in plasma PRL concentration, and broody behavior ceases (El Halawani et al., 1980). Active immunization against PRL in bantam hens decreases broodiness (March et al., 1994; Crisostomo et al., 1998), and passive immunization of the vasoactive intestinal peptide, a PRL-releasing stimulator, can be effective in preventing or interrupting broodiness (Crisostomo et al., 1997). Therefore, it is obvious that PRL or the *PRL* gene, an autosomal gene, has a role in the onset and maintenance of broodiness.

The prolactin receptor (PRLR) has an important role in the PRL signal transduction cascade, which is triggered at the onset of broodiness, and PRL exerts its biological functions by acting through the PRLR (Bole-Feysot et al., 1998). Because the *PRLR* gene is on the Z chromosome (Dunn et al., 1998), the hypothesis of sex-linked inheritance of broodiness may relate to *PRLR* gene via its role in PRL signal transduction.

Although the chicken *PRL* and *PRLR* have been cloned and mapped (Dunn et al., 1998; Ohkubo et al., 2000), information on their effects on broody behavior is lacking. The purpose of the study reported here was to evaluate the genetic effects of variation in *PRL* and *PRLR* genes on broody behavior in chickens.

MATERIALS AND METHODS

Populations

The primary stock used in this experiment was pedigreed hens (n = 155) of the Blue-shell chicken, a Chinese local breed in Jiangxi province that exhibits broodiness. Hens were reared in cages in an open-sided house on 16 h of light/d during the first part of the laying cycle. At 43 wk of age, they were transferred in groups of 19 or 20 into 8 floor pens (3 × 3 m) with wood shavings 8 to 10 cm deep. In this windowless house, each pen was equipped with 6 nesting boxes with wood shavings as nesting material. Hens were fed a commercial corn-soybean-based diet with 16.5% CP and 2,650 kcal ME/kg and had free access to feed and water. The house was automatically ventilated to maintain an ambient temperature between 20 and 28°C, with a photoperiod of 16 h light/d at 15 lx. These conditions induced some hens to exhibit broodiness, and 3 to 5 eggs were allowed to remain in each nest to promote the broody condition. The 4 wk after transferring to the windowless house was regarded as a transition period, and broody traits were not recorded during this period.

White Leghorns (n = 72), a maternal line of Hy-Line brown egg layers (n = 93), a maternal line of Avian broiler breeders (n = 20), and Chinese local breeds including Shou-guang (71), Beijing-you (n = 48), and Silkie (n = 222) were screened for allelic frequencies of the *PRL* gene. These breeds or lines, excluding Shou-guang and Beijing-you, were also screened for allelic frequencies of the *PRLR* gene. The White Leghorns have a long history of selection against broodiness, whereas this behavior is still common in Chinese local breeds.

Phenotyping for Broody Traits

Broody traits were recorded from 47 to 66 wk of age. Hens were observed from 1600 to 1700 h daily. Hens defined as broody were those that persistently nested for more than 3 consecutive days, exhibited nesting defense, and clucked characteristically.

Phenotypic traits related to broodiness included the number of nesting days, broody days, repeats of broody cycles, and duration of broodiness. Nesting days were obtained by counting all the days a hen nested during the observation period whether or not she was broody. Broody days were the total number of days of broodiness exhibited as judged by nesting and broody characteristics. Repeats of broodiness were the number of broody cycles. The criterion for the end of broodiness was that the hen did not exhibit broody behavior for more than 1 wk. Duration of broodiness was the number of days for the complete broody process including the onset and the end of broodiness. For hens that had more than one complete broody cycle, the average duration was calculated.

Genotyping for PRL and PRLR

Genomic DNA was isolated from blood samples by the phenol-chloroform method. Amplifications of fragments of the promotor of *PRL* and all the exons of *PRLR* were by PCR. Primers (Table 1) were designed according to the sequence of *PRL* (GenBank accession no. AB011434) and *PRLR* (GenBank accession no. AY237377 and AY237376) using the online primer design procedure, Primer 3.0.[3] The 20-μL PCR volume included 50 ng of DNA template, 0.20 m*M* deoxynucleoside triphosphate, 2.5 m*M* $MgCl_2$, 0.20 m*M* primer, and 0.5 U of Taq DNA polymerase.[4] The PCR protocol was 94°C for 5 min followed by 35 cycles of 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min and a final extension at 72°C for 10 min. The PCR products of *PRL* and *PRLR* were genotyped by single-stranded conformational polymorphism. Two

[3]Primer 3.0, Whitehead Institute for Biomedical Research, www.cbr.nrc.ca/cgi-bin/primer3_www.cgi.

[4]Dingguo Biotechnology Company, Beijing, P.R. China.

TABLE 1. PCR forward (F) and reverse (R) primers for the promotor of prolactin (*PRL*) and exons of the PRL receptor (*PRLR*)

Primer	Sequence	Annealing temperature (°C)	Amplicon
PRL			
PRLpro1	F: 5′-CATACTCAGCATCCCACAGC-3′		Promotor
	R: 5′-TGTTGCTCATGGTAGGGATTC-3′	58	–213 to 64
PRLpro2	F: 5′-GGTGGGTGAAGAGACAAGGA-3′		Promotor
	R: 5′-TGCTGAGTATGGCTGGATGT-3′	62	–403 to –203
PRLpro3	F: 5′-TGCCAGAAGCCTCCATTTAC-3′	60	Promotor
	R: 5′-CCTTGTCTTTCACCCACCA-3′		–503 to –385
PRLR			
PRLR1	F: 5′-CCAAAACACAGTTCACCATGA-3′	60	Exon 1
	R: 5′-AAGGAACTGCATCCCTCTTT-3′		(171 bp)
PRLR2	F: 5′-TTTTGCTCCTTGTGTTTTAGGA-3′	59	Exon 2
	R: 5′-TGGTTTCCTACCGAAAGGATT-3′		(162 bp)
PRLR3	F: 5′-TCGCATGTTTTTCAGTGAGC-3′	57	Exon 3
	R: 5′-ACCCACTGTGGTCAGAGGAA-3′		(122 bp)
PRLR4	F: 5′-CTCTCTTGCAGGACAGTCACC-3′	60	Exon 4
	R: 5′-CCTCTCACCCTTCTCTGCTG-3′		(152 bp)
PRLR4a	F: 5′-AAACCCACTGTCCAACCCTA-3′	60	Exon 4a
	R: 5′-CGCTTCTTACCTGTCCTTGC-3′		(195 bp)
PRLR5	F: 5′-TCCAGCCCTACAGAATGCTA-3′	59	Exon 5
	R: 5′-CCATTTCATTAGTTGCCCTGA-3′		(218 bp)
PRLR5a	F: 5′-TTGTCTGCTTTGATTCATTTCC-3′	59	Exon 5a
	R: 5′-TGCATTTCATTCTTCCCTTTTT-3′		(250 bp)
PRLR6	F: 5′-GCCAGATCCTCCTGTGAATG-3′	57	Exon 6
	R: 5′-TGAGGGGACATGACTAACAAA-3′		(236 bp)
PRLR6a	F: 5′-CAGTTCAGCCAGGTTCTCCT-3′	59	Exon 6a
	R: 5′-CCTCCCACTCTTCCTTTTCC-3′		(168 bp)
PRLR7	F: 5′-TTGTTGGACAGCAAACACAA-3′	58	Exon 7
	R: 5′-TTATTGAAAGGGTGCATGGTC-3′		(165 bp)
PRLR7a	F: 5′-CATAAGCATACCTCTTGGTGGTT-3′	60	Exon 7a
	R: 5′-CCACTTACCACTGGGGATCA-3′		(212 bp)
PRLR8	F: 5′-TGCATTCTGACCGTATTTCCT-3′	59	Exon 8
	R: 5′-CCATTGTCCAGCTCATGATT-3′		(141 bp)

microliters of PCR product of each individual were mixed with 5 μL of denaturing buffer (98% formamide, 0.09% xylene cyanole FF, and 0.09% bromophenol blue) and then denatured at 94°C for 5 min followed by a rapid chill on ice for 10 min. The denatured PCR products were electrophoresed for 14 h at 8 V/cm on 12% acrylamide gels. The DNA bands on the gel were stained by 0.2% $AgNO_3$ for 20 min, and then 3% Na_2CO_3 for about 5 min (Qu et al., 2005). Genotypes were recorded according to band patterns.

A 24-bp nucleotide sequence insertion was identified in the *PRLpro2* PCR product, therefore, the PCR products of *PRLpro2* were directly electrophoresed for 3 h at 16 V/cm on 12% acrylamide gels for genotyping. The PCR products of each homozygote were purified using DNA Fragment Quick Purification/Recover Kit,[5] and ligated to the pMD 18-T vector,[5] and transformed into DH5-α *Escherichia coli*[5] for PCR product cloning (Sambrook et al., 1989). Sequencing was performed on an ABI377 sequencer to identify the mutation site.

Statistical Analysis

Broody traits including the number of nesting days, broody days, repeats of broody cycles, and duration of broodiness were calculated for each genotype. Because these data (excluding repeats of broody cycles) did not conform to a normal distribution, they were transformed with $x' = \ln(x + 1)$. Genotypic effects were analyzed by 3-way ANOVA using the GLM procedure of the SAS Institute (2001) with genotype (G) as a fixed effect, and sire (s) and dam (d) as random effects, according to the following model:

$$Y = \mu + G + s + d + e$$

where Y = dependent variable, μ = population mean, and e = random error. Significant differences among least-square means of different genotypes were calculated using Duncan's multiple-range test or Bonferroni *t*-test, and

TABLE 2. Distribution of nesting days for Blue-shell hens

Nesting days	Hens (n)	Percentage
0	43	27.7
1 to 4	47	30.3
5 to 10	19	12.3
11 to 20	15	9.7
21 to 30	9	5.8
31 to 40	8	5.2
41 to 50	10	6.5
51 to 81	4	2.6
Total	155	

[5]Dingguo Biotechnology Company, Beijing, P.R. China.

TABLE 3. Frequencies and percentage of nonbroody, atypical broody, and typical broody and mean durations of broodiness for Blue-shell hens

Item	Hens (n)	Percentage[1]	Duration[2] (d)
Nonbroody	43	27.7	
Atypical broody	61	39.4	
Typical broody	51	32.9	28.8 ± 2.6
Once	36	23.2	29.4 ± 3.6
Twice	14	9.0	28.3 ± 2.5
Triple	1	0.7	17.0

[1](n/155) × 100.

[2]Means ± standard errors.

the significance determined at $P < 0.05$. Comparisons between groups for frequency of broody vs. nonbroody hens were made by a chi-squared test.

RESULTS

Incidence of Broodiness

During the observed period (140 d), the average number of nesting days and broody days were 10.5 and 12.3, respectively, for Blue-shell hens. The average repeat of broodiness was 0.43. As shown in Table 2, 27.7% of the hens did not nest; another 42.6% nested between 1 and 10 d with the remainder nesting more than 10 d.

Hens from the Blue-shell breed could be categorized into 3 groups according to the number of nesting and broody days (i.e., nonbroody, atypical broody, and typical broody; Table 3). Nonbroody hens used nests only for egg laying. Nontypical broody hens exhibited no obvious broody behavior but did exhibit sporadic nesting behavior. Typical broody was defined as hens with consecutive nesting behavior and characteristic activities associated with broodiness. During the experimental period, more than 30% of the hens showed typical broodiness, and of these about a third became broody more than once. The mean duration of broody cycle was 28.8 ± 2.6 d.

TABLE 4. Effects of prolactin promotor (*PRLpro2*) genotypes on broody traits for Blue-shell hens

	Genotype		
Item	+/+	+/−	−/−
Hens[1] (n)	9	50	88
Genotypic frequency	0.06	0.34	0.60
Nesting days[2,3]	0.73 ± 0.47[b]	1.89 ± 0.27[a]	1.55 ± 0.22[ab]
Broody days[2,3]	0 ± 0[b]	1.48 ± 0.33[a]	1.19 ± 0.26[a]
Repeats[2]	0 ± 0[b]	0.59 ± 0.14[a]	0.50 ± 0.11[a]
Duration[2,3] (d)	0 ± 0[b]	3.30 ± 0.22[a]	3.20 ± 0.17[a]
Broody frequency (%)	0	42.0	32.9
Once broody (%)	0	30.0	22.7
Twice broody (%)	0	12.0	9.1
Triple broody (%)	0	0	1.1

[a,b]Means in a row with no common superscript differ significantly ($P < 0.05$).

[1]Eight individuals among the 155 hens were not included for *PRLpro2* genotype analysis because their bands could not be correctly classified.

[2]Least-square means ± standard errors.

[3]Original data were transformed by x′ = ln (x + 1).

Polymorphisms in PRL and PRLR

Of the 15 sets of primers used to amplify the gene fragments, the loci, *PRRpro2*, *PRLR3*, and *PRLR6*, were polymorphic (Figure 1). For the *PRLpro2* locus, an insertion of 24 bp of nucleotide sequence, 5′-ACAAGAAGAGACAAGACAAGGAAG-3′, was identified. The insertion occurred in the promotor, –377 ~ –354, of *PRL* (GenBank accession no. AB011434). Three genotypes were observed: individuals with single slower, faster, and both bands were designated as genotypes +/+ (presence of the 24 bp), −/− (absence of the 24 bp), and +/− (presence/absence), respectively.

Because the *PRLR* is located on the Z chromosome, there were no heterozygous females, and 2 genotypes were identified at *PRLR3* and *PRLR6* loci. At the *PRLR3* locus, a nucleotide transition, A9026G (GenBank acces-

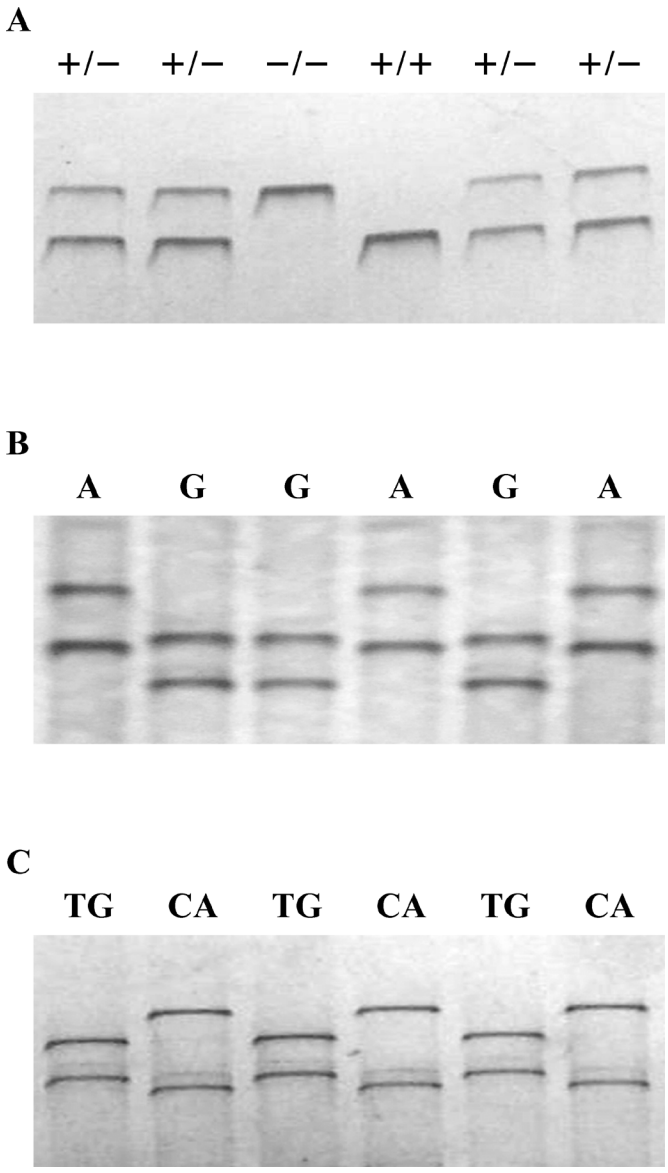

FIGURE 1. Band patterns for the 3 polymorphic loci, *PRLpro2*, *PRLR3*, and *PRLR6*. A) Genotypes of *PRLpro2*; B) genotypes of *PRLR3*; C) genotypes of *PRLR6*. PRL = prolactin; PRLR = prolactin receptor.

TABLE 5. Effects of prolatin receptor (*PRLR*) genotypes on broody traits for Blue-shell hens

	PRLR3			*PRLR6*		
Item	A	G	*P*	TG	CA	*P*
Hens[1] (n)	133	18		57	78	
Genotypic frequency	0.88	0.12		0.42	0.58	
Nesting days[2,3]	1.40 ± 0.19	1.38 ± 0.37	0.95	1.19 ± 0.27	1.59 ± 0.30	0.28
Broody days[2,3]	0.92 ± 0.23	0.85 ± 0.45	0.89	0.70 ± 0.33	1.08 ± 0.37	0.41
Repeats[2]	0.34 ± 0.10	0.39 ± 0.19	0.80	0.30 ± 0.14	0.43 ± 0.15	0.52
Duration[2,3] (d)	3.15 ± 0.10	3.35 ± 0.33	0.55	3.08 ± 0.20	3.42 ± 0.19	0.08
Broody frequency (%)	35.4	22.3		29.8	42.4	
Once broody (%)	26.3	5.6		19.3	30.8	
Twice broody (%)	8.3	16.7		10.5	10.3	
Triple broody (%)	0.8	0		0	1.3	

[1]During genotype analysis 4 individuals for *PRLR3* and 20 hens for *PRLR6* were not included because their bands could not be correctly classified.

[2]Least-square means ± standard errors.

[3]Original data were transformed by $x' = \ln(x + 1)$.

sion no. AY237377), was present in the exon 3 of *PRLR*. The single nucleotide polymorphism (SNP) did not lead to amino acid variation in that it was in 5′-untranslated region (5′-UTR) of *PRLR* cDNA. At the *PRLR6* locus, 2 SNP (T14771C and G14820A) were detected, and therefore 2 genotypes (TG and CA) were described. The T14771C SNP led to an amino acid variation, Leu340Ser (GenBank accession no. AY237376), in PRLR, whereas G14820A was a synonymous mutation.

Association of Genotypes with Broody Traits

The frequency for genotype −/− (0.60) at the *PRLpro2* locus was greater than that (0.06) for +/+ (Table 4), and the effects of *PRLpro2* genotypes on broody traits of Blue-shell hens differed ($P < 0.05$). None of the +/+ hens exhibit broodiness, whereas 42.0% of the +/− and 32.9% of the −/− hens went broody. The chi-squared test ($\chi^2 = 18.12$) showed that the incidence of typical broody of +/− and −/− was higher ($P < 0.01$) than that of +/+. Genotypes +/− and −/− did not differ for any of the broody traits.

As shown in Table 5, the frequency for genotype A (0.88) at *PRLR3* locus was greater than that (0.12) for G, whereas TG and CA at *PRLR6* exhibited approximately the same frequency. There were no differences ($P > 0.05$) between genotypes for *PRLR3* or *PRLR6* for any of the broody traits.

Frequencies of Genotypes and Alleles

Frequency distributions for genotypes and alleles varied among breeds and lines. For *PRLpro2* (Table 6), the White Leghorn was homozygous +/+, whereas the Beijing-you and the Silkie were homozygous −/−. The remaining stocks exhibited both + and − alleles with a very high frequency of − in the Shou-guang. As shown in Table 7 for *PRLR3*, the locus was fixed for genotype A in the White Leghorn, and genotype A was more frequent than G in the other breeds except for the Hy-Line. The genotype TG at *PRLR6* was fixed for the White Leghorn and more frequent than CA in the Hy-Line and Avian stocks (Table 7). The Chinese breeds shared approximately equal frequencies.

DISCUSSION

Prolactin, one of pituitary hormones, regulates important physiological functions, ranging from well-known effects in mammalian reproduction to osmoregulation in fish and nesting behavior in birds (Elkins et al., 2000). In turkeys, changes in plasma PRL levels are associated with the expression of *PRL* mRNA in the anterior pituitary (Wong et al., 1991). The expression of *PRL* depends on the 5′-flanking region sequence. Studies with mammals and birds have shown that Pit-1/GHF-1 (Kurima et al., 1995; Frisch et al., 2000), estrogen receptors

TABLE 6. Frequencies of genotypes and alleles of prolactin promotor (*PRLpro2*) in different chicken breeds or lines

		Genotype			Allele	
Breed or line	Hens (n)	+/+	+/−	−/−	+	−
White Leghorn	72	1.00	0	0	1.00	0
Hy-Line layer	93	0.13	0.42	0.45	0.34	0.66
Avian broiler	20	0.10	0.35	0.55	0.27	0.73
Blue-shell	147	0.06	0.34	0.60	0.23	0.77
Shou-guang	71	0	0.06	0.94	0.03	0.97
Beijing-you	48	0	0	1.00	0	1.00
Silkie	222	0	0.01	0.99	0	1.00

TABLE 7. Frequencies of genotypes in the prolactin receptor (*PRLR*) in different chicken breeds or lines

	PRLR3			*PRLR6*		
Breed or line	n	A	G	n	TG	CA
White Leghorn	47	1.00	0	48	1.00	0
Hy-Line layer	93	0.33	0.67	47	0.67	0.33
Avian broiler	20	0.65	0.35	20	0.94	0.06
Blue-shell	151	0.88	0.12	135	0.42	0.58
Silkie	196	0.83	0.17	202	0.53	0.47

(Maurer and Notides, 1987), and the CCAAT-enhancer binding protein-α (Day et al., 2003; Enwright et al., 2003), and other proteins are essential in regulating the expression of *PRL* via specific promotor binding sites. The sequence variation in the 5′-flanking region of *PRL* may lead to changes in transcriptional factor binding sites and alter the expression of *PRL*. In the experiment reported here, there was a 24-bp nucleotide sequence insertion at –377 ~ –354 of the 5′-flanking region of *PRL*. Insertion of this sequence in the promoter may inhibit a transcriptional factor binding site for *PRL* and, therefore, decrease the expression of PRL, which contributes to nonbroodiness in +/+ hens. For most native chicken breeds, however, natural selection should favor those hens with adequate broodiness in order to maintain normal reproduction, and the frequency for the allele of absence of the insertion was predominant in most breeds examined. Absence of the 24-bp insertion sequence would maintain normal function of the *PRL* promoter. The fact that −/− and +/− hens of the Blue-shell chicken exhibited similar broody phenotype suggested that even one normal *PRL* promoter in the heterozygotes would be sufficient for normal expression of PRL.

One SNP in the exon 3 and 2 SNP in the exon 6 of *PRLR* were identified. Mutation in *PRLR3* did not lead to amino acid changes in PRLR due to the mutation in 5′-untranslated region of *PRLR* cDNA. This may be the reason that the mutation was not associated with broodiness. At the *PRLR6* locus, the SNP of G14820A did not contribute to broodiness because it was synonymous mutation. PRL interacts with the extracellular domain of PRLR in various target cells and activate a cascade of intracellular events, mainly JAK-Stat signal transduction pathway, via specific sites of the PRLR cytoplasmic tail (Bole-Feysot et al., 1998). The T14771C in exon 6 of *PRLR*, a missense mutation, leads to Leu340Ser in PRLR. This amino acid variation occurred in the cytoplasmic tail of the PRLR and may not influence the functional structure and, therefore, did not influence broodiness, which is consistent with the view that broodiness is not controlled by a major gene on Z chromosome (Romanov et al., 2002).

The White Leghorn, Hy-Line layers, Avian broiler line, and several Chinese breeds were used as reference populations to examine distribution profiles of genotypes and alleles in *PRL* and *PRLR*. For *PRLpro2*, the White Leghorn was the only +/+ line, and the Silkie and Beijing-you the only −/− breeds. Because broodiness is seldom observed in White Leghorns, the distribution profile of *PRLpro2* is consistent with the observation for Blue-shell chickens that absence of the 24-bp insertion in *PRL* promotor is associated with the phenotypic expression of broodiness. The polymorphism for *PRLpro2* was present in the other stocks, especially in Hy-Line brown egg layer, which has been intensively selected for egg production and has a low incidence of broodiness. These results imply that other genes, in addition to *PRL*, are involved in broodiness, which is consistent with the complexity of incubation behavior involving the onset and maintenance of broodiness.

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