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ORIGINAL ARTICLE

Molecular characterization and an 80-bp indel polymorphism within the prolactin receptor (*PRLR*) gene and its associations with chicken growth and carcass traits

Ke Liang[1] · Xiangnan Wang[1] · Xiaoxiao Tian[1] · Rui Geng[1] · Wenya Li[1] · Zhenzhu Jing[1] · Ruili Han[1] · Yadong Tian[1] · Xiaojun Liu[1] · Xiangtao Kang[1] · Zhuanjian Li[1]

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Abstract

The prolactin receptor (*PRLR*), a type I cytokine receptor, must bind prolactin (*PRL)* to act on target cells to mediate various physiological functions, including reproduction and lactation. This study identified an 80-bp insertion/deletion (indel) polymorphism in the 3′-untranslated region (3′-UTR) of the chicken *PRLR* gene in 3736 individuals from 15 breeds and analyzed its associations with growth and carcass traits in an F_2 resource population. The results of the association analysis indicated that the 80-bp indel polymorphism was significantly ($P < 0.05$) or very significantly ($P < 0.01$) associated with multiple growth and carcass traits, such as body weight, leg weight, and shank length. In addition, we found that during the breeding process of commercial laying hens and commercial broilers, the 80-bp indel locus was artificially selected for the II genotype. Together, our findings reveal that this 80-bp indel polymorphism has potential as a new molecular marker for marker-assisted selection of chicken growth and carcass traits.

Keywords Prolactin receptor · Association analysis · Growth traits · Insertion/deletion · Genetic differentiation

Introduction

Over the course of the past decades, scientists have made great progress in the field of animal genetics and breeding by screening candidate genes as markers of animal productivity (Meuwissen 2007). In China, the growth rates of most local chicken breeds are low, and their production performance is poor, which restricts the development of the poultry industry

Ke Liang and Xiangnan Wang contributed equally to this work.

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✉ Zhuanjian Li
lizhuanjian@163.com

Ke Liang
kkeliang@hotmail.com

Xiangnan Wang
18838917969@163.com

Xiaoxiao Tian
tianxiaoxiao96@163.com

Rui Geng
gengrui6666@163.com

Wenya Li
1716964176@qq.com

Zhenzhu Jing
208608870@qq.com

Ruili Han
rlhan@126.com

Yadong Tian
ydtian111@163.com

Xiaojun Liu
Xjliu2008@hotmail.com

Xiangtao Kang
xtkang2001@263.net

[1] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou 450046, China

to a certain extent. To fully exploit the excellent traits of the local chicken populations and improve their production performance, it is necessary to search for major effect genes that control growth and carcass traits to support marker-assisted breeding.

The prolactin receptor (*PRLR*), a type I cytokine receptor characterized by its ability to activate associated signaling proteins such as JAK2 and STATs, is the specific receptor for prolactin (*PRL*) (Fleenor et al. 2006; Morammazi et al. 2016). *PRL*, also known as luteotropic hormone or luteotropin, is best known as a single-chain peptide hormone that enables mammals, usually females, to produce milk (Evans et al. 1989; Mihailov et al. 2014). *PRL* plays a vital role in metabolism, regulation of the immune system and pancreatic development and performs multiple functions in the central nervous system from physiology to pathology (An et al. 2015; Bole-Feysot et al. 1998; Goffin et al. 2002). To mediate the various physiological functions mentioned above, *PRLR* must bind *PRL* as a transmembrane receptor to act on target cells (Bole-Feysot et al. 1998).

In view of the important physiological functions of *PRLR* in animal reproduction and the differences in its genetic effects in numerous studies (Drogemuller et al. 2001; Linville et al. 2001; Rens et al. 2003; Vincent et al. 1998), scholars worldwide have conducted a great deal of research on polymorphisms in *PRLR*. Considering the vital role of *PRLR* in animal mammary gland development and milk synthesis, researchers have adopted the *PRLR* gene as an important candidate gene for dairy cow milking traits. In terms of growth and development, a study has shown that the S18N variation site significantly affected body height, body length, chest circumference, hip width, body weight and average daily gain in Nanyang cattle (Lü et al. 2011). In the study of sheep breeds, the *PRLR* gene has primarily been used as a candidate gene for reproductive traits, milk production and cashmere quality. In Small-Tail Han sheep, 2 polymorphic sites in *PRLR* intron 1 and 1 polymorphic site in exon 10 were identified. Further analysis showed that these different genotypes of the polymorphic loci significantly affected lambing number, and the dominant genotypes were BB and AB (Chu et al. 2011). In addition, the *PRLR* gene in domestic pigs has been studied extensively as a candidate gene for litter size. The PCR-SSCP method was used by Mihailov et al. (2014) to detect polymorphisms of the *PRLR* gene in three groups of sows: Large White, Danish Landrace (LD), and Landrace × Yorkshire × Duroc hybrids (L × Y × D). It was found that the BB genotype was associated with the largest number of viable offspring. In LD sows, the AB genotype was associated with the shortest number of days to reach 100 kg; in L × Y × D hybrids, the AA genotype was associated with better meat traits than BB genotype. At present, studies of *PRLR* gene polymorphisms in poultry are mainly focused on egg-laying performance and broodiness. In some poultry breeds, a single nucleotide polymorphism (SNP) in the exon of the *PRLR* gene has been shown to result in significant differences in egg production among different genotypes (Rashidi et al. 2012; Chen et al. 2012). However, there are few studies on the effects of *PRLR* gene variants in the growth and development of poultry.

In this study, the full-length coding sequence (CDS) region of the *PRLR* gene was analyzed using bioinformatic methods. This study aimed to simultaneously identify indel polymorphisms in the *PRLR* gene in different poultry breeds and to analyze its genetic effect on growth and carcass traits. These results will illuminate the biological roles of the *PRLR* gene in the growth and development of poultry and serve as a theoretical reference for molecular breeding of poultry.

Materials and methods

Ethics approval

All animal experiments and animal care methods were approved by the Institutional Animal Care and Use Committee (IACUC) of Henan Agricultural University, Zhengzhou, P.R. China (Permit Number: 11–0085) and were performed in accordance with the protocols outlined in the "Guide for Care and Use of Laboratory Animals" (Henan Agricultural University). Chickens were allowed access to feed and water ad libitum under normal conditions. All animal experiments and methods were carried out in accordance with the approved guidelines, and all efforts were made to minimize suffering.

Animal sampling and trait measurement

In total, 3736 chickens were used as study subjects: 797 in the F_2 resource population; 1854 from dual-purpose Chinese native chicken breeds [Gushi blue-shell chicken (GS, $n = 272$, 16 weeks), Xichuan black-bone chicken (XC, $n = 393$, 6 weeks), Changshun blue-shell chicken (CS, $n = 142$, 16 weeks), Dongxiang blue-shell chicken (DX, $n = 172$, 16 weeks), Lushi blue-shell chicken (LS, $n = 132$, 6 weeks and 14 weeks) and Wuhei chicken (WH, $n = 479$, 28 weeks)]; two cultivated lines [Xiaohuayu chicken (H-lines, $n = 170$, 72 weeks), Hengbanluhua chicken (B-lines, $n = 94$, 72 weeks)]; 704 commercial broilers [recessive white chicken (RW, $n = 96$, 6 weeks), Arbor Acres broiler (AA, $n = 169$, 1 day), Ross308 ($n = 172$, 1 day), Hubbard broiler (HBD, $n = 267$, 21 day)]; and 381 commercial hens [Lohmann brown laying hen (LB, $n = 63$, 1 day) and Hyline brown hen (HB, $n = 318$, 1 day)]. Among these populations, the breeding methods of the two cultivated lines (H-lines and B-lines) have been described previously (Wang et al. 2019a, b). The F_2

resource population was produced after two hatchings from an F_1 generation constructed via reciprocal crossing between slow-growing Chinese native GS chickens (2 roosters and 24 hens) and fast-growing Anka broilers (4 roosters and 12 hens). In total, 836 chickens from the F_2 resource population were slaughtered at 84 days of age. The growth and carcass traits of the F_2 individuals were measured for further analysis. The measuring methods and additional details were performed as described by Han et al. (2011).

DNA samples, polymerase chain reaction (PCR), and genotyping

Blood samples were collected from the jugular vein, and genomic DNA was isolated using the phenol–chloroform extraction method. The blood samples of 836 F_2 individuals were collected during slaughtering and stored at − 80 °C until further analysis. All genomic DNA samples were quantified and subsequently diluted to 50 ng/μL for use as working solutions.

According to the published Gallus *PRLR* gene sequence (NC_006127.5) in GenBank, a pair of primers for *PRLR* was designed using Oligo 7 software and synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). The primer sequence used to amplify the specific DNA fragment is shown in Table 1. The 10 μL volume PCRs contained 50 ng of template DNA, 3 μL of ultrapure water, 5 μL of 2 × *Taq* Master Mix (Kangwei, Beijing, China), and 0.5 μM of each primer. An initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 30 s, followed by annealing at 63 °C for 30 s and extension at 72 °C for 30 s; and a final extension at 72 °C for 10 min constituted the PCR amplification procedure. Subsequently, the PCR products were electrophoresed on 2.0% agarose gels stained with DNAgreen in 1 × TBE buffer. Different genotypes of the indel were confirmed by sequencing.

Table 1 Details of primer pairs

Gene	Primer sequence (5′-3′)	Tm (°C)	Size (bp)
PRLR	F: TGGGCTGCTACACTGTTGTT	63	219/139
	R: AGTGGGAACTCTGATACT GCTG		
Q-PRLR	F: GTGGGTCTGGCAGGTCAATC	60	202
	R: AGCAGGAATTGGGACCTGAT		
GAPDH	F: GAACATCATCCCAGCGTCCA	60	132
	R:CGGCAGGTCAGGTCAACAAC		

RNA isolation, cDNA preparation, and real-time quantitative PCR

Total RNA was extracted from different tissues (including the heart, liver, spleen, lung, kidney, duodenum, skin, muscular stomach, jejunum, ileum, cecum, breast muscle, and leg muscle) of 4 LS chickens aged 20 weeks and 30 weeks and treated as previously described (Li et al. 2019). A pair of specific *Q-PRLR* primers was designed to detect different gene expression levels in different tissues and stages, and its sequence is shown in Table 1. The *GAPDH* gene was selected as an internal control, and two pairs of primers for *GAPDH* were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). The composition of the real-time quantitative PCR (qPCR), the reaction conditions, and the method of calculating the relative expression have been described in a previous study (Han et al. 2019). All data are presented as the mean ± standard deviation (mean ± SD).

Bioinformatics analysis

As shown in Table S1, the mRNA and amino acid sequences of the *PRLR* gene from 16 species were searched and downloaded from the National Center for Biotechnology Information (NCBI). Using MEGA 7.0 (Molecular Evolutionary Genetics Analysis) software, the amino acid sequences from 16 species were analyzed, and a phylogenetic tree was constructed using the neighbor-joining method. A bootstrap test with 1000 replicates was used to estimate the reliability of the constructed phylogenetic tree, and Poisson correction was used in the construction of the model. DNAMAN (version 6) was used to analyze the chicken *PRLR* gene DNA, mRNA, and amino acid sequences. Moreover, the amino acid sequences of the chicken *PRLR* genes were analyzed using a series of bioinformatics websites, as shown in Table S2.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) and the allelic frequencies and genotypes of these breeds were calculated using the SHEsis program (http://analysis.bio-x.cn), and χ^2 tests performed with SPSS software (Version 24.0; NY, USA) were used to analyze the differences in frequencies among individuals. In addition, Nei's methods were used in Popgene software (Version 1.3.1) to compute the colony genetic indices, including heterozygosity (He), effective allele numbers (Ne), and polymorphism information content (PIC) (Nei and Roychoudhury 1974; Yeh 1999).

SPSS 24.0 was used to perform all the statistical analyses according to the following two linear mixed models. For growth traits, model I ($Y_{ijklm}=\mu+G_i+S_j+H_k+f_l+e_{ijklm}$) was used. In the case of the effects of body weight on carcass

traits, model II ($Y_{ijklm}=\mu+G_i+S_j+H_k+f_l+b\ (W_{ijklm}-\overline{W})+e_{ijklm}$) was applied to the association analysis of carcass traits, in which carcass weight was used as a concomitant variable. In these two models, Y_{ijklm} represents the observed value; μ is the overall population mean; G_i is the fixed effect of the genotype; S_j is the fixed effect of sex; H_k is the fixed effect of hatching; f_l is the fixed effect of family; b represents the regression coefficient for carcass weight; $\overline{W}$ represents average slaughter weight; W_{ijklm} represents individual slaughter weight; and e_{ijklm} is the random error (Ren et al. 2019). All data for each trait obtained by statistical analysis are presented as the mean ± standard error (mean ± SE). $P<0.05$ was considered to be statistically significant, and Bonferroni's test was performed to control for multiple comparisons (Li et al. 2019).

Results

Bioinformatic analysis of the chicken *PRLR* gene

The *PRLR* gene of chickens has 25 exons and is located on chromosome Z. Multiple transcript variants of the *PRLR* gene exist. The mRNA sequence (Accession: NM_204854) was chosen for bioinformatic analysis in this study. The full-length CDS region of the *PRLR* gene (Accession: MG517522.1) was 2496 bp, encoding 831 amino acids (Accession: NP_990185).

The results of physical and chemical analyses showed that the molecular weight of the *PRLR* protein (molecular formula: $C_{4166}H_{6472}N_{1106}O_{1298}S_{40}$) was 94102.25 kD, and the isoelectric point was 5.41, indicating that the protein was acidic. There are 831 amino acids in the protein, of which 84 are positively charged (Arg, Lys) and 110 are negatively charged (Asp, Glu). The total average hydrophilic index presented in Fig S1 was −0.590, suggesting that the protein was hydrophilic. The signal peptide prediction result yielded C, Y, and S values of 0.297, 0.478, and 0.910, respectively. As shown in Fig S2, this amino acid sequence has a potential signal peptide splice site located at amino acid 24, and the signal peptide length is 1–23. However, two transmembrane domains were predicted (Fig S3), the first of which (5–27) might be the signal peptide sequence. Protein phosphorylation sites were predicted, and those with scores above 0.7 are shown in Fig S4. The results of the secondary structure prediction (Fig S5) are as follows: random coil, 53.67%, 446 amino acids; extended strand, 23.83%, 198 amino acids; α helix, 17.21%, 143 amino acids; and β turn, 5.29%, 44 amino acids.

Molecular evolution and genetic conservation analysis

The genetic conserved synteny analysis shown in Fig. 1 revealed that the *PRLR* gene was highly conserved in diverse species, with a consistent position on the chromosome and transcriptional orientation, which suggested that *PRLR* has essential functions in organism. As shown in Fig. 2, the phylogenetic tree was mainly divided into two categories. Humans, rats, mice, pigs, cows, sheep, platypus, etc., can be grouped together, and chickens and ducks can be grouped together. In particular, *Xenopus laevis* was grouped separately, and these species above constitute the first category. Moreover, *Zebrafish* and *Salmo salar* were distantly related to the first category and were classified as

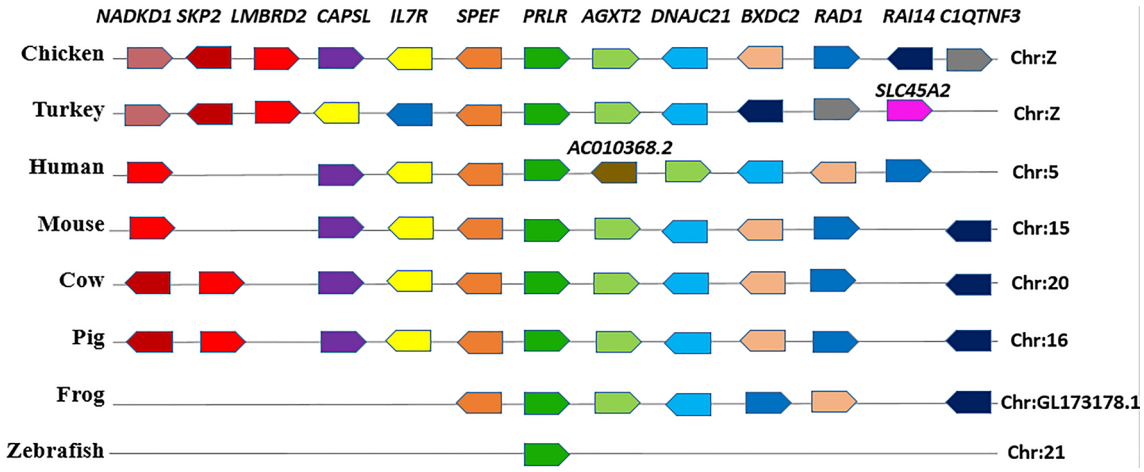

Fig. 1 Conserved genetic synteny for the genomic region of the *PRLR* gene in different species. Species and their corresponding chromosomes are listed on the left and right sides of the figure, respectively, and the gene symbols are listed at the top. Boxes of the same color represent the same gene. The transcriptional direction of each gene is shown by the direction of the gene boxes. The figure is drawn and edited by Microsoft PowerPoint 2016

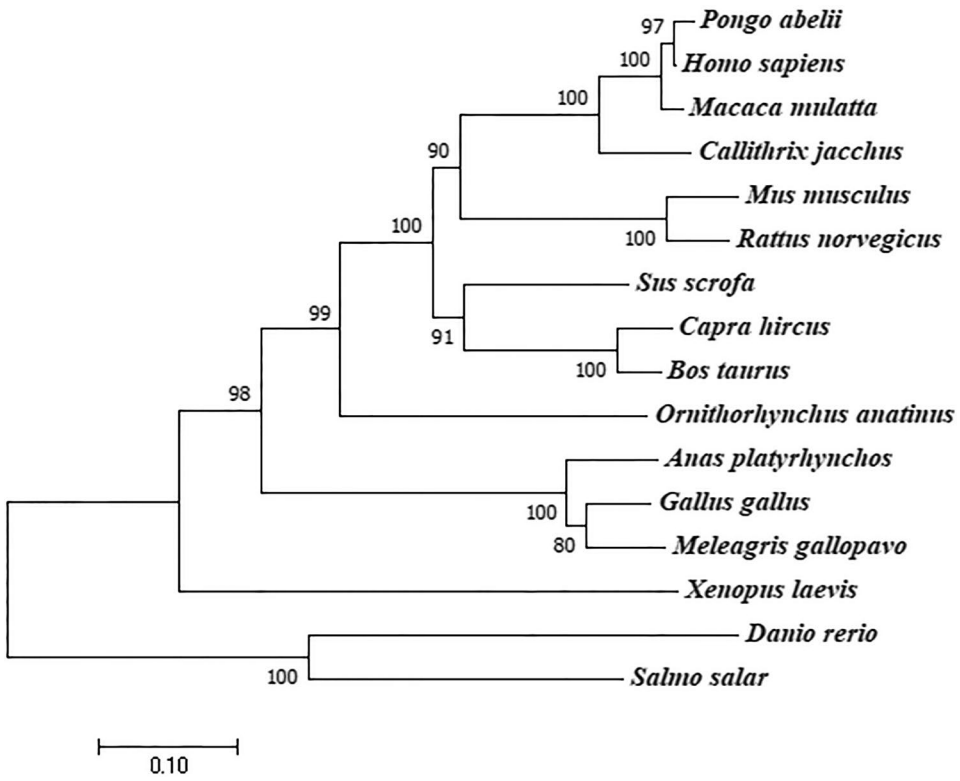

Fig. 2 Phylogenetic relationships among 16 species

the second category. Interestingly, as shown in Fig. 2 and Table S1, the mRNA sequence lengths of the *PRLR* genes of some closely related species are fairly similar; otherwise, the mRNA sequence lengths vary.

Relative expression in different tissues and ages

To further identify the physiological function of *PRLR*, the gene expression in different tissues of 20-week-old and 30-week-old LS chickens was measured by qPCR. The results in Fig. 3 show that *PRLR* gene expression in spleen and duodenum tissues was significantly higher at 30 weeks than at 20 weeks. In contrast, expression increased significantly at 20 weeks compared to that at 30 weeks in the heart and cecum tissues. Additionally, the alteration of the *PRLR* gene relative expression values between 20- and 30 weeks in the spleen and heart tissues was very significant ($P < 0.01$). In general, *PRLR* gene expression was highest in the lung, duodenum and ileum, and the tissues with the lowest expression were the breast and leg tissues.

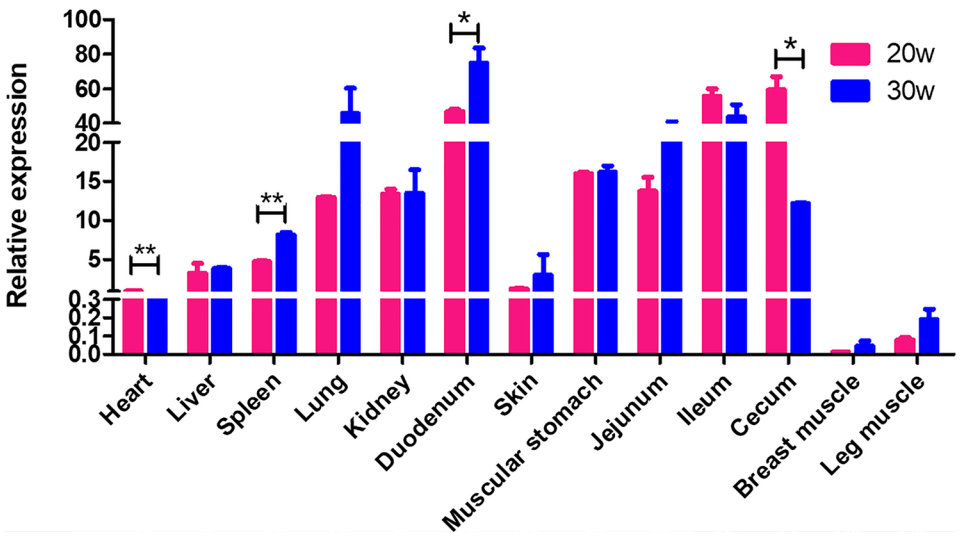

Fig. 3 Gene expression levels in different tissues of 20 weeks and 30 weeks LS chickens. * Represents a significant difference ($P < 0.05$); ** represents a very significant difference ($P < 0.01$)

Genotyping and sequencing confirmation

A novel 80-bp indel mutation in the 3′-UTR of the *PRLR* gene was detected by whole-genome resequencing and was described as NC_006127.5 g. 10604211ins cacaaatgcc actcttcatc tggaatggca cagccatcag ttttgtaaat gcattatatt tctcagagat10604290. This indel polymorphism was genotyped in the 3736 studied individuals by agarose gel electrophoresis, and three genotypes were identified, as shown in Fig. 4. The sizes of the DNA fragments of these three genotypes were as follows: the II genotype was 219 bp, the ID genotype was 219 bp and 139 bp, and the DD genotype was 139 bp. Furthermore, the different variants of the *PRLR* gene were sequenced to confirm the presence of the 80-bp indel. As shown in Fig. 5, the sequence resulting from the PCR product also confirmed that an 80-bp insertion was located in the downstream region of the *PRLR* gene.

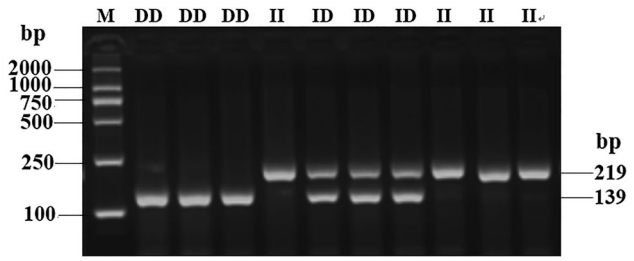

Fig. 4 Electrophoresis pattern of the 80-bp indel within the chicken *PRLR* gene

Genotypic distribution and allelic frequencies

The frequencies of the three genotypes and two alleles, as well as polymorphic indicators associated with the 80-bp indel of the *PRLR* gene in 15 breeds, were calculated and analyzed by HWE tests to identify the distribution of genotypes among different breeds. The results shown in Table 2 indicate that in Chinese native chicken breeds, the frequency of genotype II was extremely higher than that of genotype DD, except in the DX breed. Similarly, the I allele frequency was much higher than the D allele frequency. Notably, only the II genotype of the *PRLR* gene was found in commercial laying hens and broilers, which suggested that the 80-bp indel of the *PRLR* gene was artificially selected during the breeding process of these commercial chickens.

Association between the *PRLR* gene 80-bp indel and growth and carcass traits

To detect the functional effects of the 80-bp indel polymorphism of the *PRLR* gene, the association between this indel and the phenotypic traits of the F_2 resource population was analyzed. As shown in Table 3, the indel was significantly associated with carcass weight (CW), semi-evisceration weight (SEW), evisceration weight (EW), head weight

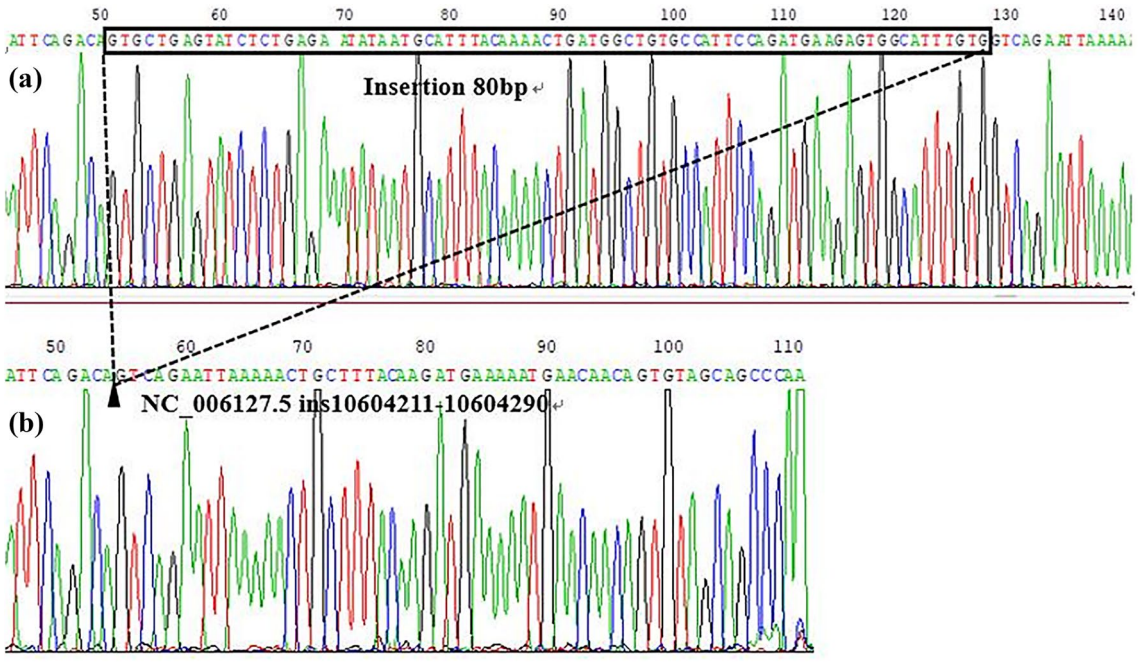

Fig. 5 Sequencing comparison of the 80-bp indel polymorphism of the chicken *PRLR* gene. The results above were obtained by reverse sequencing. **a** is the partial sequence of the I allele; **b** is the partial sequence of the D allele

Table 2 Genotypic and allelic frequencies and related genetic parameters for the chicken *PRLR* gene in 15 breeds

Breeds/n	Genotypic and allelic frequencies					He	Ne	PIC	*P*-value
	II	ID	DD	I	D				(HWE)
F_2/797	0.93	0.04	0.03	0.95	0.05	0.10	1.11	0.09	0.00
GS/272	0.93	0.02	0.05	0.94	0.06	0.11	1.12	0.10	0.00
XC/393	0.71	0.07	0.22	0.74	0.26	0.38	1.62	0.31	0.00
CS/142	0.82	0.05	0.13	0.84	0.16	0.27	1.36	0.23	0.00
DX/172	0.30	0.52	0.18	0.56	0.44	0.49	1.97	0.37	0.42
LS/132	0.74	0.06	0.20	0.77	0.23	0.35	1.54	0.29	0.00
WH/479	0.65	0.31	0.04	0.81	0.19	0.31	1.46	0.26	0.99
H-lines/170	0.97	0.00	0.03	0.97	0.03	0.06	1.06	0.06	0.00
B-lines/94	0.98	0.01	0.01	0.98	0.02	0.03	1.03	0.03	0.00
RW/96	0.85	0.15	0.00	0.93	0.07	0.14	1.16	0.13	0.44
AA/169	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	\
Ross308/172	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	\
HBD/267	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	\
LM/63	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	\
HB/318	1.00	0.00	0.00	1.00	0.00	0.00	1.00	0.00	\

F_2: F_2 generation resource population; GS: Gushi chicken; XC: Xichuan black-bone chicken; DX: Dongxiang green eggshell chicken; LS: Lushi green eggshell chicken; WH: Wuhei chicken; H-lines: Xiaohuayu chicken; B-lines: Hengbanluhua chicken; RW: recessive white chicken; AA: Arbor Acres broiler; HBD: Hubbard broiler; LM: Lohmann Brown laying hen; HB: Hyline brown; He: gene heterozygosity; Ne: effective allele numbers; PIC: polymorphism information content; *P*-value (HWE): *P*-value of Hardy–weinberg equilibrium

Table 3 Effect of the 80-bp indel of the *PRLR* gene on carcass traits in chickens

Carcass traits	Mean ± SE			*P*-value
	II	ID	DD	
CW	1210.68 ± 22.49^{ab}	1277.84 ± 43.06^{a}	1152.22 ± 41.47^{b}	0.030
SEW	1096.50 ± 20.25^{ab}	1162.88 ± 40.85^{a}	1040.78 ± 39.15^{b}	0.029
EW	916.98 ± 19.25^{ab}	964.55 ± 36.12^{a}	867.51 ± 34.73^{b}	0.049
HW	43.07 ± 0.43^{b}	46.73 ± 1.22^{a}	39.10 ± 1.16^{c}	0.000
cW	58.38 ± 1.18^{b}	64.92 ± 2.47^{a}	48.90 ± 2.37^{c}	0.000
WW	122.33 ± 2.62^{a}	125.67 ± 4.85^{a}	111.21 ± 4.68^{b}	0.010
LW	148.92 ± 3.23^{ab}	159.66 ± 5.99^{a}	139.75 ± 5.78^{b}	0.009

CW: carcass weight; SEW: semi-evisceration weight; EW: evisceration weight; HW: head weight; cW: claw weight; WW: wing weight; LW: leg weight. Different lowercase letters (superscript) indicate significant differences in the means ($P < 0.05$); the same letters indicate no difference ($P > 0.05$)

(HW), claw weight (cW), wing weight (WW) and leg weight (LW) ($P < 0.05$), and ID genotype individuals had the highest values for these carcass traits. Furthermore, among these carcass traits, HW, cW, WW and LW were strongly correlated with the 80-bp indel ($P < 0.01$).

An analysis of the effect of this indel on chicken growth traits was performed (Table 4), and interestingly, body weight (BW), shank length (ShL), shank girth (SG), sternal length (StL), and body slanting length (BSL) were significantly different ($P < 0.05$) at the ages of 8 weeks and 12 weeks. In addition, the effects of the indel genotypes on phenotypic traits were observed, and the results show that the values of these traits were highest in ID individuals. The difference between the ID and DD genotypes was significant ($P < 0.05$), while the difference between the ID and II genotypes was not significant ($P > 0.05$).

Discussion

Recently, genetic variation has been observed both in nucleotide sequences and in the length of coding sequence (Zhou et al. 2015). However, compared to SNPs, indel variants have higher efficiency and wider application because of their advantages of convenient detection and strong effects (Li et al. 2017). Genome size can also be affected by the relative

Table 4 Effect of the 80-bp indel of the *PRLR* gene on growth traits in chickens

Grow traits	Age (week)	Mean ± SE			*P*-value
		II	ID	DD	
BW (g)	0	30.64 ± 0.44	31.26 ± 0.72	31.19 ± 0.70	0.429
	2	122.42 ± 2.38	118.38 ± 4.66	119.85 ± 4.55	0.119
	6	559.76 ± 11.90	591.25 ± 21.63	543.22 ± 21.09	0.112
	8	811.72 ± 15.94^{b}	884.15 ± 31.41^{a}	777.01 ± 31.39^{b}	0.008
	10	1109.63 ± 21.22	1154.07 ± 40.12	1056.64 ± 38.98	0.086
	12	1347.20 ± 22.32^{ab}	1431.26 ± 47.31^{a}	1298.44 ± 45.50^{b}	0.045
ShL (cm)	4	5.49 ± 0.05	5.51 ± 0.16	5.48 ± 0.15	0.986
	8	7.90 ± 0.04^{ab}	8.11 ± 0.14^{a}	7.66 ± 0.14^{b}	0.045
	12	9.39 ± 0.05^{b}	9.74 ± 0.14^{a}	8.88 ± 0.13^{c}	0.000
SG (cm)	4	2.69 ± 0.02	2.71 ± 0.05	2.67 ± 0.05	0.783
	8	3.42 ± 0.02^{a}	3.49 ± 0.05^{a}	3.29 ± 0.05^{b}	0.006
	12	3.84 ± 0.02^{a}	3.97 ± 0.06^{a}	3.71 ± 0.05^{b}	0.001
StL (cm)	4	6.21 ± 0.05	6.32 ± 0.12	6.10 ± 0.12	0.287
	8	8.90 ± 0.06^{ab}	9.14 ± 0.15^{a}	8.58 ± 0.15^{b}	0.010
	12	10.98 ± 0.07^{ab}	11.24 ± 0.16^{a}	10.65 ± 0.16^{b}	0.009
BSL (cm)	4	11.38 ± 0.09	11.46 ± 0.19	11.35 ± 0.18	0.864
	8	16.24 ± 0.08^{a}	16.28 ± 0.25^{a}	15.64 ± 0.25^{b}	0.041
	12	19.77 ± 0.07^{ab}	20.12 ± 0.23^{a}	19.29 ± 0.22^{b}	0.019

BW: body weight; ShL: shank length; SG: shank girth; StL: sternal length; BSL: body slanting length. Different lowercase letters (superscript) indicate significant differences in the means ($P < 0.05$); the same letters indicate no difference ($P > 0.05$)

incidence of indels, which have been recognized as a key parameter governing genome size evolution. In addition, due to their usage in phylogenetic reconstruction and their essential role in genomic evolution, indels are attracting increasing interest (Paśko et al. 2011). The fact that indel variation plays crucial roles in many aspects of animal growth and carcass traits has been revealed by related studies (Wang et al. 2019a, b). In this study, an 80-bp indel polymorphism in the 3′-UTR of the chicken *PRLR* gene was revealed for the first time.

In the artificial breeding process of commercial laying hens, particular attention is paid to reproductive traits, while the selection of commercial broilers focuses on meat quality traits. Therefore, along with the genetic changes caused by population differentiation that occurred during the domestication process, various beneficial alleles have become fixed in these specialized commercial breeds. We can conclude that during the poultry domestication process and in the recent development of breeds and commercial production populations, artificial selection has shaped the amount and distribution of genetic variation (Jia et al. 2016). Remarkably, the results of this study indicated that only II genotypes were identified in almost all commercial laying hens and broiler chickens, suggesting that during the breeding process of these commercial chickens, the 80-bp indel of the *PRLR* gene was artificially selected, leading to the appearance of obvious genetic differentiation.

As mentioned above, due to their effects on economic value (Gaya et al. 2006), the growth and carcass traits of chickens are the main reference indicators for molecular breeding. An 80-bp indel genetic variation in the chicken *PRLR* gene and its association with various growth and carcass traits in an F_2 generation resource population are described in this study. Our results indicate that the 80-bp indel polymorphism is significantly or very significantly associated with multiple growth and carcass traits, such as BW, SL, and LW. Interestingly, this study revealed that the associations between the indel and BW, ShL, SG, StL, and BSL were significant ($P < 0.05$) at the ages of 8 weeks and 12 weeks but not at the age of 4 weeks or younger. At different ages, the hormone expression levels in chickens change to adapt to biological behavior that occurs during specific periods. For example, LS chickens enter the laying period at 20 weeks, and that period peaks at 30 weeks. Based on these results, we can speculate that the effect of the 80-bp indel polymorphism of the *PRLR* gene on growth and carcass traits at different ages is influenced by changes in *PRL* expression levels during specific growth stages. The association analysis showed that for most of the phenotypic traits, the values of the ID genotype individuals were highest, and the differences between the ID and DD genotypes were significant ($P < 0.05$), which illustrated that the I allele was the dominant allele. This study demonstrates that this indel polymorphism has great

potential as a molecular marker to eliminate or choose preferred individuals in the breeding of local chickens. In terms of the relative expression of the *PRLR* gene, gene expression in different tissues and stages was analyzed, and the results suggested that chicken *PRLR* gene expression was highest in the lung, duodenum and ileum, whereas the tissues with the lowest expression were the breast muscle and leg muscle. In addition, the results of genetic conservation analysis revealed that the *PRLR* gene was conserved in diverse species but may have functional differences between mammals and nonmammals. Previous research has shown that in accordance with the various actions of *PRL* in vertebrates, *PRLR* is widely expressed in diverse tissues, including the brain, ovary, placenta, and uterus (Binart et al. 2010; Bole-Feysot et al. 1998). However, unlike *PRLR* gene expression in mammals, which is controlled by multiple promoters (Hu et al. 1998; Zhang et al. 2002), *PRLR* is also widely expressed in various tissues of avian species, such as chickens (Ohkubo et al. 1998) and pigeons (Chen and Horseman 1994). Moreover, we found that *PRLR* gene expression in the lung, duodenum and jejunum tissues was much higher at 30 weeks than at 20 weeks. Conversely, *PRLR* gene expression was greater at 20 weeks than at 30 weeks in the ileum and cecum. This result may indicate that the expression of *PRLR* changes as the expression of *PRL* changes in different tissues before and after the laying period, thereby better exerting its physiological functions. However, it is necessary to perform a further functional analysis of *PRLR* at the molecular level to gain a better understanding of the biological function and regulatory mechanisms of *PRLR* in chickens.

In summary, the chicken *PRLR* mRNA was characterized at the molecular level using bioinformatics methods. We found that during the breeding process of commercial laying hens and broiler chickens, the 80-bp indel had been artificially selected, leading to obvious genetic differentiation. Importantly, this study revealed for the first time that the 80-bp indel polymorphism of the *PRLR* gene is significantly associated with growth and carcass traits in chickens. Taken together, these results indicate that the *PRLR* gene can be used as a candidate gene for not only reproductive traits but also growth and carcass traits in chickens.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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