Polymorphism of Ghrelin Gene in Twelve Chinese Indigenous Chicken Breeds and Its Relationship with Chicken Growth Traits

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ABSTRACT : A 2,656 bp fragment of chicken ghrelin gene was cloned and SNPs were detected by PCR-RFLP and Allele Specific PCR (ASP) in 12 Chinese indigenous chicken breeds and a commercial chicken population. The results showed that there were 23 base variations and an amino acid change (Gln→Arg) in cloned chicken ghrelin gene. Three SNPs were confirmed in 13 populations and associations between this gene and growth traits of Tibetan chicken (TC) and Recessive White chicken (RW) were investigated. The results of haplotype analysis revealed that 26 haplotype genotypes were composed of eight haplotypes. The results of x^2 tests indicated that there were significant differences between genotypes or haplotype genotype frequencies in some of the breeds or sexes at 0.05 or 0.01 levels. The results of ANOVA revealed that there were significant differences between genotypes or haplotype genotypes on some growth traits of TC and RW chicken breeds at 0.05 or 0.01 levels. Multiple comparisons showed that there were significant associations between genotype CT at site 71 and some growth traits of two chicken breeds and between genotype AG at site 1,215 and body weight at 16 wk of two chicken breeds, and there was a significant association between haplotype genotype CAA/CAG and body weight and shank girth at 16 wk of two chicken breeds. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 2 : 153-159)***

Key Words : Ghrelin Gene, SNP, ASP/SNAP, Association Analysis, Chinese Indigenous Chicken Breeds

INTRODUCTION

Kojima et al. (1999) firstly purified and identified the coding protein of ghrelin gene (GHR) in rat stomach. It is an endogenous ligand for growth-hormone secretagogues receptor (GHS-R). This ligand, which mainly expresses in mammal stomach and can specifically stimulate pituitary for releasing growth hormone (GH), has a strong effect on function and metabolism of stomach, gut and heart (Kojima et al., 1999; Ahmed et al., 2002; Noritoshi et al., 2003). This hormone increases food intake and accelerates growth rate (Guido et al., 2002; Iglesias et al., 2004; Pecker et al., 2004). Kaiya et al. (2001) cloned chicken ghrelin gene. The mRNA sequence of chicken ghrelin gene consists of 843 base pairs and encodes 26 amino acids and it has five exons and four introns. Kaiyai's (2002) and Wada's (2003) studies showed that chicken ghrelin gene is primarily present in the proventriculus but absent in the gizzard. At the same time, low levels of expression were also detected in brain, lung, and intestine. But its function was not different from mammals'. And it could increase not only plasma GH levels but also plasma corticosterone levels (Geelissen et al., 2003). Furuse et al. (2001 and 2002) illuminated that intracerebroventricular injection of ghrelin and growth hormone releasing factor in different doses inhibited food intake in neonatal chicks in the different degree. However ghrelin gene had influence on chicken hormone levels, physiological circumstances and growth and development, previous studies were focused on the aspects of biology and biochemistry without exception but not the aspects of genetics, selection and evolution.

Both Richards (2003) and Kaiya (2004) reported that there were four bases variations in chicken ghrelin gene at four positions, but more details of the associations between ghrelin gene and chicken growth traits have not been well investigated. In order to further study this gene, we detected SNPs of ghrelin gene in 12 Chinese indigenous chicken breeds by Allele Specific-PCR (ASP) or called Single Nucleotide Amplified Polymorphisms (SNAP) and conducted associations between ghrelin gene and chicken growth traits.

MATERIALS AND METHODS

Sampling and DNA isolation

A total of 599 Chinese indigenous chicken individuals and 96 commercial chicken individuals were sampled from 12 Chinese indigenous chicken breeds and one commercial breed. The characteristics of twelve local chicken breeds are described in Poultry Genetic Resources in China (Chen et al., 2004). They were randomly collected from the pure

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Received March 3, 2005; Accepted July 7, 2005

Table 1. The main geographical distribution of 12 Chinese indigenous chicken breeds and a commercial breed

Number	Breed name	Abbreviation	Type	Locations	Number of individuals		
					Male	Female	Total
1	Silkies	WG	Medicine	Jiangxi province	18	23	41
2	Beijing fatty	YJ	Dual purpose	Beijing city	1	24	25
3	Luyuan	LY	Dual purpose	Jiangsu province	15	24	39
4	Chahua	CH	Dual purpose	Yunnan province	0	24	24
5	Xiaoshan	XS	Dual purpose	Zhejiang province	1	20	21
6	Gushi	GS	Dual purpose	Henan province	19	26	45
7	Chinese fighting	DJ	Fancy	Henan province	15	20	35
8	Dagu	DG	Dual purpose	Liaoning province	0	36	36
9	Langshan	LS	Dual purpose	Jiangsu province	3	24	27
10	White earlobes yellow	BE	Egg	Jiangxi province	14	19	33
11	Xianju	XJ	Egg	Zhejiang province	20	21	41
12	Tibetan chickens	TC	Dual purpose	Tibet	64	168	232
13	Recessive white	RW	Meat	France	40	56	96

Table 2. Polymorphisms primers and control primers of ghrelin gene at three sites

Sites	Primer names	Primer sequences	Mutation bases	Tm (°C)	PCR products size (bp)
71	R-PL1	5'-TTTTGCCAGTTTTCCTCTGTAATAC-3'	C	59.0	369
	R-PR1	5'-CTAGAGCCAGCCAGAGCAGTTT-3'			
	W-PL2	5'-TTTTGCCAGTTTTCCTCTGTAAGTT-3'	T	59.0	369
	W-PR2	5'-CTAGAGCCAGCCAGAGCAGTTT-3'			
1215	R-PL3	5'-TGAAGGGTATGTAGAAAAGGACATG-3'	G	59.0	320
	R-PR3	5'-GGACAAGGGCACTAGAGGATAATTT-3'			
	W-PL4	5'-TGAAGGGTATGTAGAAAAGGACAGA-3'	A	59.0	320
	W-PR4	5'-GGACAAGGGCACTAGAGGATAATTT-3'			
2355	R-PL5	5'-TATCTTTTGCCTTTTTAGAAACTTA-3'	A	56.0	228
	R-PR5	5'-GCCTACACGTCAGCCTGTGAT-3'			
	W-PL6	5'-TATCTTTTGCCTTTTTAGAAACTAG-3'	G	59.0	228
	W-PR6	5'-GCCTACACGTCAGCCTGTGAT-3'			
Control	C-PL0	5'-TGAAGCAACAAAGGTAAC-3'	For all	55.0	395
	C-PR0	5'-TTCTGTCCCAAGGATG-3'			

breeding farms of 12 provinces in China, and came from natural mating population. These Chinese indigenous chicken breeds are from five different ecotypic zones in China according to their evolutionary origin, geographic distribution, figure, color and product performance. Dagu (DG) chicken belong to North East zone; Beijing fatty (YJ), Gushi (GS) and Chinese fighting (DJ) chicken belong to Huanghuai sea zone; Silkies (WG), Luyuan (LY), Xiaoshan (XS), Langshan (LS), White earlobes yellow (BE) and Xianju (XJ) chicken belong to South East zone; Chahua (CH) chicken belong to South West zone and Tibetan chicken belong to Tibetan Plateau zone (Zheng et al., 1988; Chen et al., 2004). RW chicken which was bred for many years purchased from a commercial company in France. And more details about breeds name, abbreviations, the types, locations and the number of males and females are given in Table 1. Genomic DNA was presented by the leaders of Poultry Science Institute in Jiangsu Province of China and/or extracted from blood with ordinary phenol/chloroform/isopropyl-alcohoi methods (Sambrook et al., 2001).

0.5 ml blood was collected from the vein of the wing of every bird and mixed with 0.2 ml anticoagulant and 5 ml SDS-EDTA liquid, and then stored at -20°C for DNA extraction later. All chickens had freely fed and watered. Seventeen trait values of RW and TC individuals were recorded during their growth and development, and those traits were body weight, shank length and shank girth at birth; body weight, shank length and shank girth at 2 wk; body weight, shank length and shank girth at 4 wk; body weight, shank length, shank girth and body length at 7 wk; body weight, shank length, shank girth and body length at 16 wk, respectively. The measure methods of the data could found in Bao's book (1990).

Primer design, ghrelin gene cloning and sequencing and ASP/SNAP amplification

One pair primers were designed by primer premier 5.0 (PREMIER Biosoft International Co., Palo Alto, CA) for cloning chicken ghrelin gene by PCR method. Accession numbers of sequences used for primers design in this study are AY303688, AB158617, NM_001001131, AY299454 and AB075215. SNP detection was conducted by PCR-RFLP (Wang et al., 2004; Zeng et al., 2005) and ASP/SNAP (Drenkard et al., 2000; Gregory et al., 2003). ASP primers

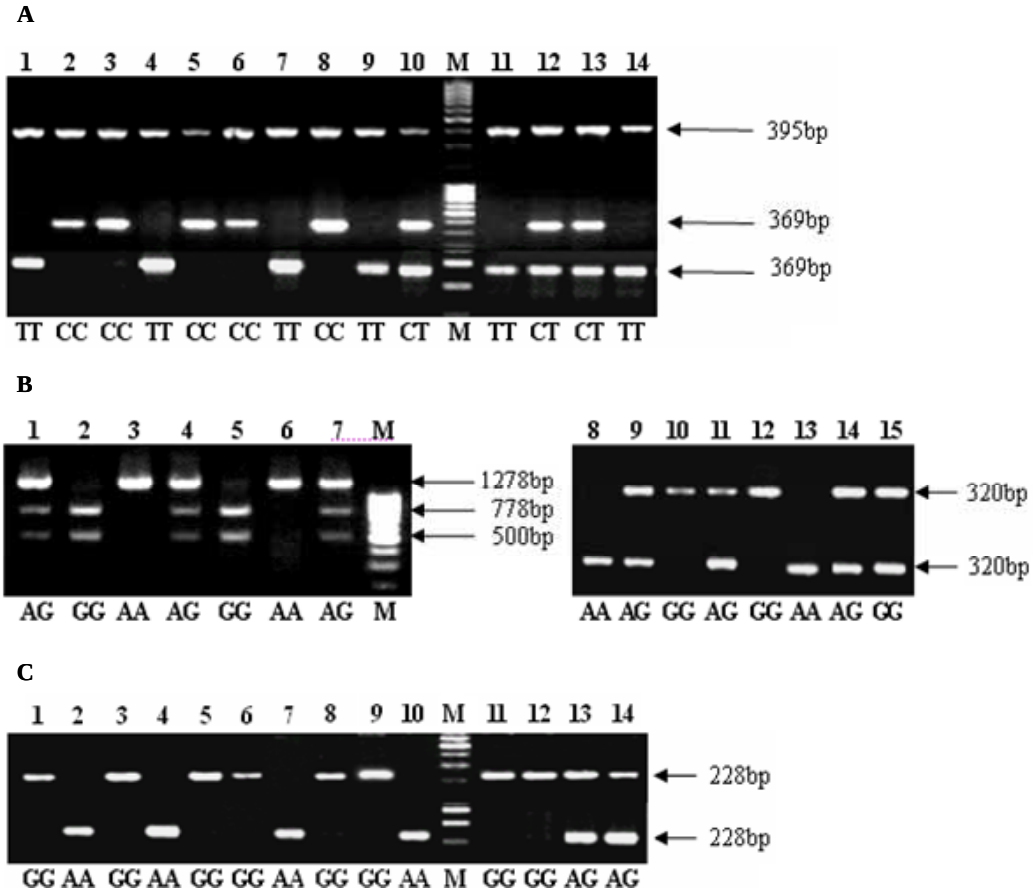

Figure 1. Polymorphic pictures of chicken ghrelin gene at three sites. A. The ASP polymorphic pictures of PCR products at site 71. Up: positive control; Middle: allele71-C; Down: allele71-T. B. The *Eco72* I-RFLP and ASP polymorphic pictures of PCR products at site 1,215. Left: The *Eco72* I-RFLP picture; Right: The ASP polymorphic pictures (Up: allele 1,215-G; Down: allele 1,215-A). C. M-ASP polymorphic pictures of PCR products at site 2,355. Up: allele 2,355-G (228 bp); Down: allele 2,355-A (228 bp).

for detecting polymorphisms were designed by SNAPER Program at website (http://ausubellab.mgh.harvard.edu/). The primers for detecting SNPs were shown in Table 2. The primers F-AAG GTG GCT GTT CCA TAA GG (5'-3') and R-TGC AAA TAA AGA GTG AGG GGA CC (5'-3') were used to amplify the fragment (1,278 bp) containing site 1215, followed by digestion with *Eco72* I (Restriction Endonuclease, Fermentas. MBI. Co., China) and 1.5% agarose gel electrophoresis for determining SNP.

PCR reactions for both cloning and SNP detection contained 1.5 mM 10×Buffer (with $MgCl_2$), 1 U *Taq* polymerase (Takara Biotech. Co., Dalian, China), 0.3 mM dNTP (Promega, Shanghai, China.), 10 pmol/ml of allele specific primers and 50 ng of DNA in a total volume of 20 μl. Thermal cycling was done in a MJ PT-100 (MJ Research, Waltham, Mass.) thermal cycler. The PCR profile was 94°C for 5 min to activate the DNA polymerase, followed by 34 cycles of 94°C for 40 s, annealing for 50 s, 72°C for 1 min and a final 72°C extension for 10 min. Whereas, the ASP/SNAP's time for denaturing, annealing and extension were 20 s, 20 s and 30 s, respectively. PCR products were cloned into pGEM-T Easy system (Promega Co. Shanghai, China.) and sequenced (Bioasia Biotech. and Unitgene Co., Shanghai, China).

Analysis bio-soft packages

BLAST analysis was conducted at website http://www.ncbi.nlm.nih.gov/BLAST/. At the same time, multiple sequences alignments were performed by the MegAlign program of Lasergene Version 6.0 (DNAStar) software. A GLM for association between gene and chicken growth traits was constructed: $Y_{ijkl} = \mu+B_i+G_j+S_k+I_l+e_{ijkl}$, ($\mu$: Means; B_i: Breeds effects; G_j: Genotypes or Haplotype genotypes effects; S_k: Sexes effects; I_l: Interactions effects; e_{ijkl}: error). Statistical analysis of the data of the genotypes/haplotypes was done by SPSS Version 11.5 (SPSS Inc.) and PHASE Version 2.1 software (M. Stephens, University of Washington).

RESULTS

Sequence of chicken ghrelin gene

The similarity scores of all sequences are up to 97% or higher. All these sequences were assembled into a contig by

Table 3. Genotype frequencies and allele frequencies of ghrelin gene at site 71, 1,215 and 2,355 in 13 chicken breeds

No.	Breeds	Site 71					Site 1,215					Site 2,355				
		Genotype frequencies			Allele frequencies		Genotype frequencies			Allele frequencies		Genotype frequencies			Allele frequencies	
		TT	CT	CC	T	C	AA	AG	GG	A	G	AA	AG	GG	A	G
1	WG	0.68 (28)	0.15 (6)	0.17 (7)	0.76	0.24	0.37 (15)	0.39 (16)	0.24 (10)	0.56	0.44	0.20 (8)	0.20 (8)	0.60 (25)	0.29	0.71
2	YJ	0.96 (24)	0.00 (0)	0.04 (1)	0.96	0.04	1.00 (25)	0.00 (0)	0.00 (0)	1.00	0.00	0.00 (0)	0.16 (4)	0.84 (21)	0.08	0.92
3	LY	0.69 (27)	0.18 (7)	0.13 (5)	0.78	0.22	0.72 (28)	0.28 (11)	0.00 (0)	0.86	0.14	0.51 (20)	0.15 (6)	0.34 (13)	0.59	0.41
4	CH	0.58 (14)	0.25 (6)	0.17 (4)	0.71	0.29	0.96 (23)	0.04 (1)	0.00 (0)	0.98	0.02	0.71 (17)	0.17 (4)	0.12 (3)	0.79	0.21
5	XS	0.90 (19)	0.10 (2)	0.00 (0)	0.95	0.05	0.96 (20)	0.04 (1)	0.00 (0)	0.98	0.02	0.57 (12)	0.19 (4)	0.24 (5)	0.67	0.33
6	GS	1.00 (45)	0.00 (0)	0.00 (0)	1.00	0.00	0.60 (27)	0.27 (12)	0.13 (6)	0.73	0.27	0.62 (28)	0.22 (10)	0.16 (7)	0.73	0.27
7	DJ	0.89 (31)	0.05 (2)	0.06 (2)	0.91	0.09	0.80 (28)	0.17 (6)	0.03 (1)	0.89	0.11	0.69 (24)	0.29 (10)	0.02 (1)	0.82	0.18
8	DG	0.92 (33)	0.02 (1)	0.06 (2)	0.93	0.07	0.81 (29)	0.19 (7)	0.00 (0)	0.90	0.10	0.72 (26)	0.22 (8)	0.06 (2)	0.83	0.17
9	LS	0.41 (11)	0.48 (13)	0.11 (3)	0.65	0.35	0.96 (26)	0.04 (1)	0.00 (0)	0.98	0.02	0.56 (15)	0.37 (10)	0.07 (2)	0.74	0.26
10	BE	0.73 (24)	0.15 (5)	0.12 (4)	0.80	0.20	0.64 (21)	0.36 (12)	0.00 (0)	0.82	0.18	0.42 (14)	0.36 (12)	0.22 (7)	0.61	0.39
11	XJ	0.63 (26)	0.32 (13)	0.05 (2)	0.79	0.21	0.37 (15)	0.49 (20)	0.14 (6)	0.61	0.39	0.49 (20)	0.37 (15)	0.14 (6)	0.67	0.33
12	RW	0.55 (53)	0.16 (15)	0.29 (28)	0.63	0.37	0.67 (64)	0.27 (26)	0.06 (6)	0.80	0.20	0.51 (49)	0.27 (26)	0.22 (21)	0.65	0.35
13	TC	0.51 (119)	0.29 (67)	0.20 (46)	0.66	0.34	0.59 (136)	0.34 (80)	0.07 (16)	0.76	0.24	0.43 (100)	0.25 (59)	0.32 (73)	0.56	0.44

The data in the brackets is number of individuals with this genotype.

Seqman program of Lasergene Version 6.0 (DNAStar). And it covers 97.7% of the sequence of the chicken ghrelin gene (AY303688).

SNPs detection

After BLAST and MegAlign, 23 potential SNPs sites of chicken ghrelin gene (AY303688) were found, and three of them were confirmed, they were 71 (T→C), 1,215 (G→A) and 2,355 (A→G, This nucleotide substitution results in an alteration of Codon 113 from CAG to CGG, which leads to an amino acid change from Gln to Arg), respectively (The first nucleiotide of AY303688 was defined the first one of cloned chicken ghrelin gene). An *Eco72* I-RFLP existed in site 1,215, and this site was also detected by means of ASP/SNAP. ASP/SNASP polymorphisms were identically present in site 71 and 2,355. The electrophoresis photographs were given in the following Figure 1A, B, C.

Genotype frequencies and allele frequencies of ghrelin gene

TT genotype frequencies of ghrelin gene at site 71 in 13 chicken breeds were dominantly high. The value (0.96) of TT genotype frequency in YJ chicken was highest and the value (0.5129) of TT genotype frequency in TC chicken was lowest. The allele frequencies of 71-T were higher than that of 71-C in 13 chicken breeds.

Besides WG and XJ chicken, AA genotype frequencies of ghrelin gene at site 1,215 in 11 chicken breeds were high, whereas, GG genotype frequencies were quite low, under 0.25 in these chicken breeds. Genotype AG and GG were not detected in YJ chicken. The allele frequencies of 1215-A were higher than that of 1,215-G in 13 chicken breeds.

The distribution of genotype frequencies and allele frequencies of ghrelin gene at site 2,355 were approximately parallel to that of this gene at site 1,215 except that WG and YJ chicken breeds were contrary. Genotype AA of ghrelin gene at site 2,355 in YJ chicken was not present (Table 3).

At the same time, based on the result of haplotypes analysis (which were the integrated results of three sites of chicken ghrelin gene), 26 haplotype genotypes which were present in practical experiments consisted of 8 haplotypes (Table 4). The frequency (45.75%) of haplotype TAA in all individuals was highest, and that value (1.73%) of haplotype CGA was lowest. In addition, the frequency (29.68%) of haplotype genotype TAA/TAA was higher than that value (0.14%) of CGA/TGA.

Table 4. Frequencies of haplotypes and haplotype genotypes of ghrelin gene in 13 chicken breeds

Haplotype genotypes	Frequencies	Haplotypes	Frequencies
CAA/CAA	4.47%	CAA	10.30%
CAA/CAG	1.73%	CAG	4.97%
CAA/CGA	1.44%	CGA	1.73%
CAA/CGG	1.87%	CGG	7.85%
CAA/TAA	6.63%	TAA	45.75%
CAG/CAG	1.87%	TAG	18.59%
CAG/CGG	2.16%	TGA	3.75%
CAG/TAA	1.87%	TGG	7.06%
CAG/TAG	0.43%		
CGA/CGG	0.72%		
CGA/TAA	1.15%		
CGA/TGA	0.14%		
CGG/CGG	0.72%		
CGG/TAA	3.89%		
CGG/TAG	4.18%		
CGG/TGA	0.29%		
CGG/TGG	1.15%		
TAA/TAA	29.68%		
TAA/TAG	9.37%		
TAA/TGA	4.03%		
TAA/TGG	5.19%		
TAG/TAG	9.51%		
TAG/TGG	4.18%		
TGA/TGA	1.30%		
TGA/TGG	0.43%		
TGG/TGG	1.59%		

x^2 tests of genotype frequencies of ghrelin gene

The total x^2 values for evaluating differences of the frequencies of all genotypes of ghrelin gene at site 71, 1,215 and 2,355 in 13 chicken breeds were 122.531 ($p = 0.000<0.01$), 111.801 ($p = 0.000<0.01$) and 126.811 ($p = 0.000<0.01$) ($df = 26$), respectively. And the comparison results of the frequencies of three genotypes of ghrelin gene at any one of three sites between any two of 13 chicken breeds indicated that there were significant differences at 0.05 or 0.01 levels between some of them, too. The detail information of x^2 tests of all genotype frequencies of ghrelin gene at three sites between any two chicken breeds can be obtained via correspondence with author.

Association analysis between breeds or genotypes/haplotype genotypes and growth traits

The results of the Tests of Between-Subjects Effects of ANOVA revealed that there were significant associations between several considered factors (Breeds, Sex, and Genotypes/Haplotype genotypes) and some growth traits of TC and RW chicken breeds. The higher related coefficient-R Squared also reflected this results. The results of Tests of Between-Subjects Effects revealed that there were significant differences between genotypes or haplotype genotypes on some growth traits of two chicken breeds at 0.05 or 0.01 levels. The results of single site analysis indicated that there were significant associations between genotypes of ghrelin gene at site 71 and body weight ($p = 0.045$) and shank length ($p = 0.048$) at 2 wk, body weight ($p = 0.001$) and shank girth ($p = 0.000$) at 7 wk and body weight ($p = 0.000$), shank length ($p = 0.012$) and shank girth at 16 wk ($p = 0.000$) of two chicken breeds; there were significant associations between genotypes of ghrelin gene at site 1,215 and body weight at 16 wk ($p = 0.001$), uniquely; But there were no significant associations between genotypes of ghrelin gene at site 2355 and any recorded growth traits ($p>0.001$). In addition, the results of haplotype genotypes analysis illuminated that there were significant associations between the haplotype genotypes of ghrelin gene and chicken body weight ($p = 0.000$) and shank girth ($p = 0.029$) at 16 wk.

The results of multiple comparisons (Table 5) displayed that the following facts: there were significant differences between genotype CT and CC or TT at site 71 on those growth traits of TC and RW chicken; there were significant differences between genotype AG and AA or GG at site 1,215 on body weight at 16 wk; And there were significant differences between haplotype genotype CAA/CAG and others on body weight and shank girth at 16 wk. These results implied that genotype CT at site 71, AG at site 1,215 and haplotype genotype CAA/CAG played important roles in growth and development of two chicken breeds.

DISCUSSION

In this study, we successfully applied ASP/SNAP method to detect three SNPs of ghrelin gene in 13 chicken

Table 5. The results of multiple comparisons between three genotypes of ghrelin gene at two sites in TC and RW chicken breeds

Sites	Genotypes	Body weight at 2 wk	Shank length at 2 wk	Body weight at 7 wk	Shank girth at 7 wk	Body weight at 16 wk	Shank length at 16 wk	Shank girth at 16 wk
71	CC	60.415 ± 1.715^{b}	30.809 ± 1.715^{b}	218.414 ± 8.456^{aB}	5.194 ± 0.097^{aB}	830.872 ± 23.401^{C}	82.422 ± 1.393	7.589 ± 0.126^{aB}
	CT	54.854 ± 1.618^{a}	29.918 ± 1.618^{a}	186.916 ± 7.979^{A}	4.640 ± 0.091^{A}	557.352 ± 22.080^{A}	79.887 ± 1.315^{A}	6.781 ± 0.119^{A}
	TT	58.820 ± 1.117^{b}	30.608 ± 1.117^{b}	222.486 ± 5.509^{aB}	5.035 ± 0.063^{aB}	751.574 ± 15.245^{B}	84.605 ± 0.908^{B}	7.299 ± 0.082^{aB}
1215	AA	63.712 ± 0.850	30.945 ± 0.184	237.604 ± 4.420	5.289 ± 0.048	754.343 ± 15.474^{aB}	87.243 ± 0.731	7.690 ± 0.062
	AG	60.692 ± 1.281	30.617 ± 0.277	225.863 ± 6.661	5.160 ± 0.072	658.143 ± 21.255^{A}	84.748 ± 1.102	7.359 ± 0.093
	GG	66.072 ± 2.874	31.017 ± 0.621	239.533 ± 14.941	5.121 ± 0.161	719.808 ± 46.655^{aB}	88.205 ± 2.472	7.653 ± 0.209

Mean within a column with no common superscript capital letters differed significantly ($p<0.01$) and mean within a column with no common superscript lowercases differed significantly ($p<0.05$).

breeds. The advantage of this method is that it can detect different alleles by two-round PCR without using restriction enzymes and performing complicated steps. And furthermore, associations of ghrelin gene with chicken growth traits were done.

The frequencies of genotype TT and allele 71-T at site 71, genotype AA and allele 1,215-A at site 1,215, genotype AA and allele2,355-A at site 2,355 and haplotype genotype TAA/TAA of ghrelin gene in most of 13 chicken breeds were significantly higher than the frequencies of other genotypes or haplotype genotypes, with only two or three exceptions (Tables 3 and 4). The results of x^2 tests of all genotype frequencies of ghrelin gene at three sites in 13 chicken breeds showed that there were significant differences between some of any two of 12 Chinese indigenous chicken breeds at 0.05 or 0.01 levels. Especially, there were significant difference at 0.01 levels between RW chicken breeds and most of 12 Chinese indigenous chicken breeds except WG, YJ, LY and BE chicken breeds. The significant difference between different individuals or breeds possibly came from species evolution, population differentiation and gene mutation. And they provide convenience for association between ghrelin gene and chicken growth traits and finding advantaged or disadvantaged genotypes of chicken ghrelin gene.

The results of ANOVA confirm that there were significant associations between three factors (breeds, genotype/haplotype genotypes and sex) and growth traits of TC and RW chicken breeds, but there were no significant associations between their interaction effects and those traits. In fact, of all factors, breed had a first effect on poultry traits, the second was genotype/haplotype genotypes and the third was sex.

The results of the Tests of Between-Subjects Effects of ANOVA revealed that there were significant associations between genotypes (CT at site 71 and AG at site 1,215) or haplotype genotype (CAA/CAG) and some growth traits of TC and RW chicken breeds ($p<0.05$ or 0.01). The results of multiple comparisons between genotypes or haplotype genotypes (Table 5) showed that there were significant associations between genotype CT at site 71 and some growth traits of two chicken breeds and between genotype AG at site 1,215 and body weight at 16 wk, and there was a significant association between haplotype genotype CAA/CAG and body weight and shank girth at 16 wk. The results seemly suggested that genotype CT at site 71, genotype AG at site 1,215 and haplotype genotype CAA/CAG had important functions on growth and development of TC and RW chicken. Perhaps, the reason is that existence of these SNPs influence on splicing and express of ghrelin gene and its functional exertion in chickens. And furthermore, the results suggest that the SNPs in ghrelin gene are possibly potential molecular markers for detecting growth and development of Chinese indigenous chicken population. However, it will still require our more work in bigger populations to prove that, subsequently.

ACKNOWLEDGEMENTS

This project was supported by the 2001-361 Plan of National Ministry of Agriculture of Introduced from International Agricultural Advanced Science Technology (948) and the Key Project of National Basic Research and Developmental Plan (G2000016103) of China. We thank Dr. S. H. Zhao for suggestions to this paper.

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