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Association of GH Polymorphisms with Growth Traits in Goose

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Abstract: In this study, the exons polymorphism of Growth Hormone (GH) gene were detected by Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) and DNA sequencing method in 109 individuals from Shitou goose, Wanxi white goose and Zi goose. About 5 pairs of primer were designed according to the *GH* gene sequence of goose then the association of polymorphism of *GH* gene with the growth traits was studied. The results indicated there were two nucleotide mutations at P2 and P4 locus, respectively which resulted in 10 genotypes and 3 genotypes. The χ^2-test showed that the genotype distributions of *GH* gene were in agreement with Hardy-Weinberg equilibrium in P2 and P4 locus ($p>0.05$), the difference was extremely significant in P2 locus on the distribution of genotype for Shitou goose and Zigoose while for Shitou goose, Wanxi white goose and Zi goose, it was extreme significant at P4 locus but there was no difference for the other locus. The least square on genotypes and groups analysis showed that there were significant differences ($p<0.01$) on growth traits in different groups however, only P4 locus have genotypes effect, the average value of AA genotype was the highest therefore, AA genotype could be the favorable marker for early breeding selection for high product trait of local goose.

Key words: Goose, growth hormone, PCR-SSCP, polymorphism, genetic marker, China

INTRODUCTION

Growth Hormone (GH) is an anabolic hormone synthesized and secreted by the somatotroph cells of the anterior lobe of the pituitary in a circadian and pulsatile manner (Ayuk and Sheppard, 2006), the pattern of which plays an important role in postnatal longitudinal growth and development, tissue growth, lactation, reproduction as well as protein, lipid and carbohydrate metabolism (Akers, 2006; Ayuk and Sheppard, 2006; McMahon *et al*., 2001; Ohlsson *et al*., 1998; ThidarMyint *et al*., 2008). GH effects on growth was observed in several tissues, including bone, muscle and adipose tissue. In addition, many studies was carried out in ruminants and confirmed the role of GH in regulation of mammary growth (Akers, 2006; Sejrsen *et al*., 1999, 2000).

So far, the genomic structure of the *GH* gene has been studied in chicken, duck and goose. These animals share a similar gene structure containing 5 exons and 4 introns. *GH* gene polymorphisms have also been observed in other poultry and animals.

A recent study on dairy cattle demonstrated that an MspI polymorphism at intron 3 of bovine *GH* gene was linked to the content of milk protein. About 2 polymorphic sites were found in intron 2 of Laiyin goose *GH* gene and were significant differences on egg-layingtest. Studies of genetic polymorphisms at the nucleotide level brought a promising way to improve growth traits, especially by making conventional breeding much more powerful and efficient. This will be achieved primarily by making possible the identification at birth of interesting genotypes before performance recording and incorporation of marker information in conventional selection.

Therefore, this study used PCR-SSCP method to detect genetic variation of all exon of *GH* gene in three local goose (Shitou goose, Wanxi White goose and Zi goose) and the aim of the present study was to show the effect of body weight on growth traits and to investigate genes that are potentially associated with this changes.

MATERIALS AND METHODS

Zi goose (N = 50). Wan-xi White Goose (N = 59) and shitou (N = 64) goose was obtained from Gene Pool of National Waterfowl (Taizhou) and reared under the same environment and management.

At 10 weeks of age, the blood samples were taken from the wing vein, heparin sodium was used as an anticoagulant. Genomic DNA utilized in PCR amplification was extracted by routing phenol-chloroform method. The rTaq polymerase, dNTP, primer were from

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Sangon Bioengineering (Shanghai) company and the others were from Baosheng Bioengineering (Dalian) Co. Ltd.

Primers design: About 5 primers were designed and conducted PCR according to the GH gene sequence of duck in Gen bank database (accession number AB158760), the primer sequence and PCR product size was shown in Table 1.

PCR amplification and SSCP analysis: PCR reactions were performed in (Eppendorff) according to following program: initial denaturation for 5 min at 94°C and then 34 cycles (94°C 30 sec; 51-62°C 30 sec; 72°C 50 sec) and final extension for 10 min at 72°C. The PCR reaction mix in a total volume of 20 μL contained: 10×PCR reaction buffer (including Mg^{2+}), 1.0 μL of dNTP mix (2.5 mM), 0.2 μL of Taq DNA polymerase (5 U μL^{-1}), 0.5 μL of forward primer (5 pmol L^{-1}), 0.5 μL of reverse primer (5 pmol L^{-1}), 1.0 μL of cDNA template (100 ng μL^{-1}), 14.8 μL of ddH_2O. The product of each amplification was analyzed by electrophoresis on 1% agarose gel (5 V cm^{-1}), 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2EDTA), using ethidium bromide staining (1 μg mL^{-1}).

For SSCP analysis, 2 μL PCR products were mixed with 5 μL denaturation solution (95% formamide, 0.5 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to PAGE (10%) (200×125×1.00 mm) in 1×TBE buffer and constant voltage (110 V) for 12 h. The gel was stained with 0.1% silver nitrate. The PCR products from different SSCP genotypes were sub-cloned to T-vector (Promega) and sequenced by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd, Shanghai, China. For the same genotype, 3 samples from different individuals were sequenced independently at least.

Statistical analysis: All data were analyzed by ANOVA procedure of the statistical software SPSS version 17.0. The association between GH gene and productive traits of three goose breeds were analyzed by linear model and calculated using the least square method. A fixed model was adopted according to the factors that affect phenotypic traits by using the following equation:

$$Y = \mu+B+G+G\times B+e$$

Where:

Y = Trait determination
μ = The average value of the group
G = The genotype effect
G×B = Genotype-group interaction effect
e = Random effect of residual variance

Table 1: Primers used in PCR amplification

Primer	Sequence 5' -3' flanking region	PCR product size (bp)	Ann. temp (°C)
P_1	F:GTAGCACCATTGCGAACA		
Exon1	R:GGCAATCCTCGTAAACTGA	240	57
P_2	F:TCCTCTCCACTTTGCTG		
Exon2	R:GTGGGAAGCCGTGGTAT	254	60
P_3	F:TGGGTGATTTGGGATGTCTC		
Exon3	R:TGAAGTGCTCACAGATGGAA	210	58
P_4	F:ACATTACAGAACACCTCACC		
Exon4	R:TCCTACTGCGACTTACCCT	231	59
P_5	F:CTGCCCCACTTTTTAGAGC		
Exon5	R:GCGGTAGCGGGTTTATTC	361	52

RESULTS AND DISCUSSION

The PCR amplification of goose *GH* gene encoding region: About 5 primers were designed to amplify the 5 exon sequence. The results showed there was specific and clear band for each exon, respectively, the PCR product could be used for PCR-SSC P analysis.

Detection of the goose GH genetic polymorphism

SSCP detection of *GH* gene exon: PCR-SSCP results showed the genetic polymorphism was detected only at exon 2 and 4. For exon 2 there were 4 alleles (named A, B, C and D) and 10 genotypes were observed (Fig. 1), defined AA, BB,CC, DD, AB, AC, AD, BC, BD and CD genotype, respectively. For exon 4 there were 2 alleles (named A and B) and 3 genotypes were detected (Fig. 2), defined AA, BB and AB genotype, respectively.

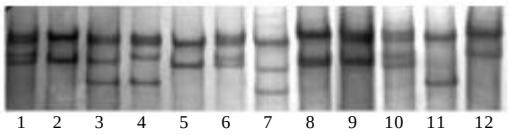

Fig. 1: SSCP analysis of PCR products of the exon 2 of *GH* gene (11: AA; 2: BB; 12: CC; 5: DD; 3: AB; 4: AC; 7: AD; 8: BC; 9: BD; 1, 6, 10: CD)

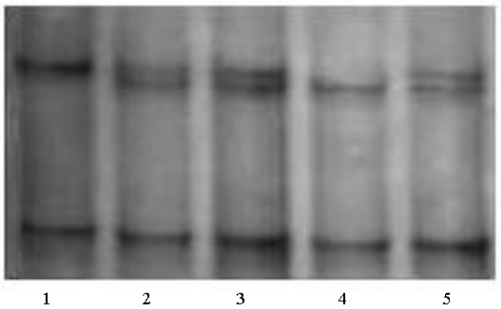

Fig. 2: SSCP analysis of PCR products of the exon 4 of *GH* gene (1: AA; 4: BB; 2, 3, 5: AB)

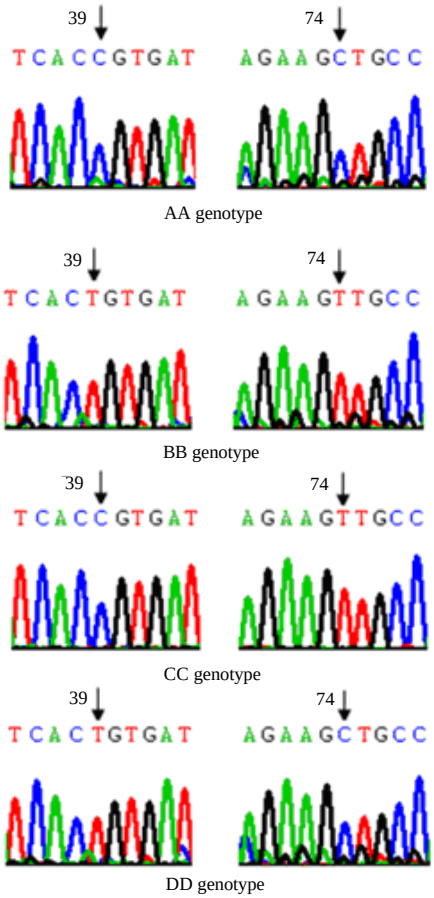

Fig. 3: Polymorphisms in the exon 2 of *GH* gene

Sequencing of different GH exon homozygous genotype: DNA sequencing analysis showed the sequence of AA genotype at exon 2 was the same to the sequence (AB158760) in Gene bank, two novel SNP (Fig. 3) was found in the BB genotype.

According to the mutation nomenclature (www.genomic.unimelb.edu.au/mdi/mutnomen or www.hgvs.org/mutnomen) and its extensions and suggestions (Den Dunnen and Antonarakis, 2000), the mutations were described as c.39 T>C and c.74 T>C which would result in synonymous mutation at 39 bp however, there was just one mutation found at CC (c.39 T>C) and DD genotype (c.74 T>C), respectively which resulted in the missense mutation (Ala→Val) at 74 bp. For the exon 4, the sequence of AA genotype was the same to the sequence (AB158760) in Gene bank, two novel SNP was found in BB genotype, c.291 C>T and c.297C>G (Fig. 4) which all resulted in synonymous mutation.

Genotypes and allele frequency in different goose group: The genotype and alleles frequency was shown in Table 2. For the P2 locus, the dominant alleles was A and

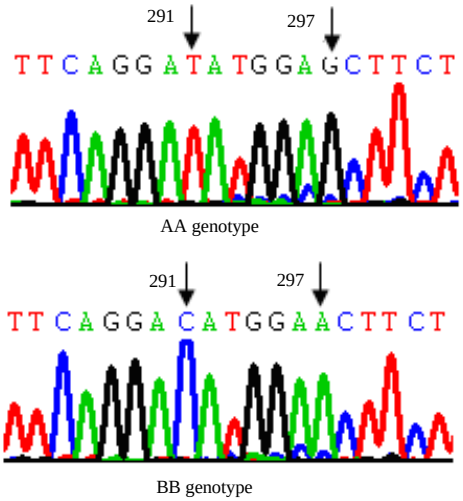

Fig. 4: Polymorphisms in the exon 4 of *GH* gene

Table 2: Genotypes and allele frequency distribution in different goose group

Charactaristics	Shitou goose (64)	Wan-xi white goose (59)	Zi goose (50)
Genotypes frequency			
AA	0.2031 (13)	0.1186 (7)	0.1 (5)
BB	0.0313 (2)	0.0169 (1)	0 (0)
CC	0.0780 (5)	0.1355 (8)	0.08 (4)
DD	0.1094 (7)	0.1864 (11)	0.24 (12)
AB	0.1406 (9)	0.0678 (4)	0 (0)
P2 locus			
AC	0.25 (16)	0.1864 (11)	0.06 (3)
AD	0.0469 (3)	0.1356 (8)	0.16 (8)
BC	0.0156 (1)	0.0339 (2)	0.04 (2)
BD	0.0469 (3)	0.0508 (3)	0.02 (1)
CD	0.0781 (5)	0.0678 (4)	0.26 (13)
Allele frequency			
A	0.4219	0.3136	0.2188
B	0.1328	0.0932	0.0312
C	0.2500	0.2797	0.2708
D	0.1953	0.3136	0.4792
p	9.2300	5.84	3.34
Genotypes frequency			
AA	0.4688 (30)	0.2033 (12)	0.24 (12)
BB	0.1406 (9)	0.2203 (13)	0.32 (16)
AB	0.3906 (25)	0.5763 (34)	0.44 (22)
P4 locus (allele frequency)			
A	0.6641	0.4915	0.46
B	0.3359	0.5085	0.54
p	0.62	0.69	0

$\chi^2_{0.05\,(2)} = 5.99$, $\chi^2_{0.01\,(2)} = 9.21$;1:$\chi^2_{0.05\,(9)} = 16.92$; (number in the bracket means number of genotypes)

Table 3: χ^2 test of genotypes distribution in different goosegroups

Locus	Groups	Shitou goose	Zi goose
P2	Wan-xi white goose	9.09	8.77
	Zi goose	30.37**	-
P4	Wan-xi white goose	9.63**	2.15
	Zi goose	12.61**	

$\chi^2_{0.05\,(2)} = 5.99$, $\chi^2_{0.01\,(2)} = 9.21$ $\chi^2_{0.05\,(9)} = 16.92$, $\chi^2_{0.01\,(9)} = 21.67$

D for three goose breeds. The χ^2-test showed that the genotype distributions at P2 locus was in agreement with Hardy-Weinberg equilibrium ($p>0.05$). For the P4 locus, B allele showed the highest frequency at Shitou

Table 4: Effect of the source of variation on the value of body weight

	F-value							
	P2 locus (weeks)				P4 locus (weeks)			
Source	6	7	8	9	6	7	8	9
Group	66.62**	92.91**	82.11**	85.33**	138.23**	180.34**	138.08**	169.03**
Genotype	0.77	0.91	0.81	0.57	6.72**	5.65**	4.96**	5.38**
Group-genotype	1.36	1.65	1.20	0.85	1.78	1.35	1.08	1.55

p<0.05, **p<0.01

Table 5: Comparison of body weight value in different groups

Items (weeks)	Shitou goose	Wan-xi white goose	Zi goose
6	2159.92±36.82^a	1837.68±42.32^b	1243.33±29.15^c
7	2662.14±42.65^a	2341.49±50.64^b	1473.38±32.60^c
8	3051.20±54.51^a	2572.38±55.95^b	1766.80±38.08^c
9	3547.13±64.45^a	2994.98±58.87^b	1990.04±39.41^c

The same superscripts in the same line means not significant difference (p>0.05), different superscripts means significant difference (p<0.01)

goose group then B allele was the dominant allele; A allele was the highest at Wan-xi white and Zi goose group so it was the dominant allele. The χ^2-test showed that the genotype distributions at P4 locus was in agreement with Hardy-Weinberg equilibrium (p>0.05). P2 and P4 were used to amplify different exon region then leaded to the different genotype for three different goose population. It was clear to show from Table 3, the distributions of the P2 locus were significantly different (p<0.01) among the goose populations except for Zi goose and Wan-xi white goose (p>0.05). The distributions of the P4 locus were significantly different (p<0.01) except for Zi goose and Shitou goose (p>0.05).

Least square analysis of the genotype and the body weight: In this study, the association of the *GH* gene polymorphism with the body weight was shown (Table 4), the result showed that the group effect of the body weight was significantly different (p<0.01) in the two locus among the group, the genotype effect of that was significantly different (p<0.01) in P2 locus. The effect of genotype-group interaction was not significantly different (p>0.05) but P4 locus had no genotype effect (p>0.05). In addition, the body weight of different breeds was analyzed statistically using analysis of variance and compared by way of Duncan in this study (Table 5). The results indicated the body weight of every week of age was significantly different (p>0.05) in the three goose group and the general trend was Shitou goose> Wan-xi white goose> Zi goose.

That of Shitou goose was the highest and greater than Wan-xi white goose and Zi goose (p<0.01). The genotype effect of the body weight at P4 locus was shown in Table 6 using the least squares. In the three goose groups, the general trend of body weight was AA> BB> AB but the AA genotype was >BB genotype in Zi goose group.

Usually, candidate gene was one method to determine whether specific genes are significantly associated with performance traits in livestock. The candidate gene approach was justified when genes previously identified in species of interest or in other species have functions related to the traits of interest (Yu *et al*., 1995). For obvious reasons, several researchers had investigated growth hormone effects in cattle and pigs (Nielsen and Larsen, 1992). In this study, the single-strand conformation polymorphism method was used to identify polymorphism in the *GH* gene including regions from exon 1-5. About 2 polymorphisms were detected in exon 2 and 4 of the *GH* gene. The polymorphisms genetic variations in exon 2 had 10 genotypes but the polymorphisms genetic variations in exon 4 had three genotypes which was novel polymorphisms. Genotypic frequencies were not found to be significantly different among three local goose based on χ^2-test which illustrated that the three local goose had a low selection effect. Hence, it suggested that heredity structure was quite stable in these three goose groups and selection or genetic drift had little effect on them. Genotype distribution in different local goose was significantly different between Zi goose and Shitou goose in exon 2 locus however, both Zi goose and Wanxi white goose was significantly different with Shitou goose in exon 4 locus. The discrepancy may caused by selection of natural and geographical environment and the selection direction of variety of different options for the beginning. In addition, this result could be limited by the sample size since Zi goose was small-sized breed for laying egg, Wanxi white was medium size breed and Shitou goose was the largest population in china local goose. Growth Hormone (GH) is necessary for tissue growth, fat metabolism and homeorhesis thus, it play an important role in reproduction, lactation and normal body growth (Burton *et al*., 1994; Ohlsson *et al*., 1998).

Growth hormone gene polymorphism in White Leghorn, Bantamised WLH and Bantam birds selected for egg production and its correlation with age at first egg, body weights and egg production. There were significant (p<0.05) differences among the three breed groups for body weights, age at first egg and egg production (Kuhnlein *et al*., 1997). In this study, researchers assessed

Table 6: Least square means standard and deviation of different genotypes in P4 locusfor body weight

Breeds	Genotype	6 weeks	7 weeks	8 weeks	9 weeks
Shitou goose					
	AA	2254.57±56.400^{a}	2775.27±57.020^{a}	3186.90±73.920^{a}	3716.10±85.35^{a}
	BB	2182.56±83.170^{a}	2660.67±125.00^{a}	3043.56±161.20^{a}	3517.33±214.2^{a}
	AB	2038.20±50.940^{b}	2526.92±65.050^{b}	2891.12±83.280^{b}	3355.08±64.45^{b}
	AA	1998.25±103.32^{a}	2475.75±118.44^{a}	2745.42±136.04^{a}	3256.45±98.62^{a}
Wan-xi white goose					
	BB	1958.92±63.930^{a}	2476.62±72.430^{a}	2724.15±77.970^{a}	3082.77±100.23^{a}
	AB	1734.65±53.580^{b}	2236.25±68.890^{b}	2236.25±74.220^{b}	2880.20±81.53^{b}
	AA	1336.45±75.080	1584.00±74.910	1842.42±91.750^{b}	2079.17±87.48^{b}
Zi goose					
	BB	1161.56±40.950	1386.00±48.250	1708.81±58.190^{a}	1851.44±60.56^{a}
	AB	1256.86±29.150	1476.59±47.330	1767.73±57.110^{a}	2042.23±56.05^{b}

The same superscripts in the same line means not significant difference ($p>0.05$), different superscripts means significant difference ($p<0.05$)

the interaction among *GH* gene polymorphism and different groups and body weight in Chinese local goose at 6-9 weeks of age. To the knowledge, the present findings genotypes and groups had an great effect on body weight but there was no interactive effect between genotypes and groups. The body weight in different group analysis showed that there were significant difference among three goose.

The average of Shitou goose body weight was the largest, the second one was Wanxi white goose body weight which was larger than Zi goose. Then Analysis of genotypes for exon 2 and 4 and its correlation with body weight showed that exon 4 presented significant difference on body weight at 6-9 weeks. There were no significant differences in the 6-9 weeks body weight among the different genotype at exon 2 locus. The genotypes at exon 2 locus did not affect body weight. The average of body weight for the AA genotype was significantly higher than those for the BB and AB genotypes in exon 4 locus.

Therefore, AA genotype groups was the best groups for the selection which can increase the growth rate. It can draw a conclusion from this study that GH could be a candidate gene for a quantitative trait locus in goose.

CONCLUSION

This study was conducted to study the polymorphism of *GH* gene in goose. There were two nucleotide mutations at P2 and P4 locus, respectively which resulted in ten genotypes and three genotypes. The χ^2 test showed that the genotype distributions of *GH* gene were in agreement with Hardy-Weinberg equilibrium in P2 and P4 locus ($p>0.05$). The least square on genotypes and groups analysis showed that there were significant differences ($p<0.01$) on growth traits in different groups however, only P4 locus have genotypes effect, the average value of AA genotype was the highest therefore, AA genotype could be the favorable marker for early breeding selection for high product trait of local goose.

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REFERENCES

Akers, R.M., 2006. Major advances associated with hormone and growth factor regulation of mammary growth and lactation in dairy cows. J. Dairy Sci., 89: 1222-1234.

Ayuk, J. and M.C. Sheppard, 2006. Growth hormone and its disorders. Postgrad. Med. J., 82: 24-30.

Burton, J.L., B.W. McBride, E. Block, D.R. Glimm and J.J. Kennelly, 1994. A review of bovine growth hormone. Can. J. Anim. Sci., 74: 167-201.

Den Dunnen, J.T. and S.E. Antonarakis, 2000. Mutation nomenclature extensions and suggestions to describe complex mutations: A discussion. Hum. Mutat., 15: 7-12.

Kuhnlein, U., L. Ni, S. Weigend, J.S. Gavora, W. Fairfull and D. Zadworny, 1997. DNA polymorph isms in the chicken growth hormone gene: Response to selection for disease resistance and association with egg production. Anim. Genet., 28: 116-123.

McMahon, C.D., R.P. Radcliff, K.J. Lookingland and H.A. Tucker, 2001. Neuroregulation of growth hormone secretion in domestic animals. Domestic Anim. Endocrinol., 20: 65-87.

Nielsen, V.H. and N.J. Larsen, 1992. Study of the association of production traits with haplotypes of the growth hormone gene in pigs. Anim. Genet., 23: 105-105.

Ohlsson, C., B.A. Bengtsson, O.G. Isaksson, T.T. Andreassen and M.C. Slootweg, 1998. Growth hormone and bone. Endocr. Rev., 19: 55-79.

Sejrsen, K., S. Purup, M. Vestergaardm and J. Foldager, 2000. High body weight gain and reduced bovine mammary growth: Physiological basis and implications for milk yield potential. Domestic Anim. Endocrinol., 19: 93-104.

Sejrsen, K., S. Purup, M. Vestergaard, M.S. Weber and C.H. Knight, 1999. Growth hormone and mammary development. Domestic Anim. Endocrinol., 17: 117-129.

ThidarMyint, H., H. Yoshida, T. Ito, M. He, H. Inoue and H. Kuwayama, 2008. Combined administration of ghrelin and GHRH synergistically stimulates GH release in Holstein preweaning calves. Domestic Anim. Endocrinol., 34: 118-123.

Yu, T.P., C.K. Tuggle, C.B. Schmitz and M.F. Rothschild, 1995. Association of PIT1 polymorphisms with growth and carcass traits in pigs. J. Anim. Sci., 73: 1282-1288.