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Association of 5-Flanking Region of *Pit-1* Gene Polymorphisms with Growth Traits in Goose

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Abstract: The aim of the present study was to detect Single Nucleotide Polymorphism (SNP) in some areas of *Pit-1* gene of goose to find genetic marker influencing on growth and carcass trait. The Single Nucleotide Polymorphism (SNP) of *Pit-1* gene promoter was investigated in Wanxi geese. The primers for promoter in *Pit-1* gene were designed according to the *Pit-1* gene promoter of goose and the SNPs were detected by PCR-SSCP method. The results showed that only C-930T was detected for SNP and two genotypes (CC and TT) were found, respectively. The analysis of least square on genotypes and groups showed that there were significant differences ($p<0.01$) on body weight of different weeks in different groups and genotypes, however, there were no differences ($p>0.05$) on body weight of different weeks in genotype-group interaction effect, then, comparison of bodyweight in different groups show that the average body weight of wanxi white goose was the largest, the average body weight of taihu goose was larger than zi goose. Variance analysis on genotype suggested that the average body weight of CC genotype and TT genotype had significant differences ($p<0.05$) in 6, 8 weeks and had extremely significant differences ($p<0.05$) in 10 weeks. This study indicated that the polymorphic site could be a genetic marker for growth trait.

Key words: Goose, Pit-1, Single Nucleotide Polymorphism (SNP), polymorphism, genetic marker

INTRODUCTION

Pit-1 is one of the key regulators of gene expression in the anterior pituitary gland. The presence of Pit-1 is an essential and determining factor in the differentiation of the somatotrope, lactotrope and thyrotrope cell lineages (Augustijn *et al*., 2002).

Pit-1 is a pituitary-specific POU-domain DNA binding factor which binds to and transactivates promoters of Growth Hormone (GH), Prolactin (PRL) and Thyroid-Stimulating Hormone-β (TSHβ) encoding genes (Ingraham *et al*., 1988; Bodner *et al*., 1988; Steinfelder *et al*., 1992) and the pituitary-specific transcription factor gene (*Pit-1*) itself in animal anterior pituitary (McCormick *et al*., 1990; Sornson *et al*., 1996). The POU domain, named after the proteins POU1F1, OCT-1/OCT-2 and UNC86 which share a common domain, POU1F1 is conserved among species and is responsible for binding to the promoters of target genes (Van As *et al*., 2000; Weatherly *et al*., 2001). *Pit-1* gene promoter regions is unkonw in livestock and poultry thus single nucleotide polymorphisms analyse mainly in exon and intron, however, many results show that promoter regions bases of mutation, insert and deficiency play a key role in genetic transcription (Liang *et al*., 2006; Wong *et al*., 2008; Fan *et al*., 2010), so the genetic variation of *Pit-1* promoter regions has important significance on gene regulation. In the present study, 5-flanking region of *Pit-1* gene has been cloned and sequenced using Chinese native geese (Zi goose, Taihu goose and Wanxi white goose). The researchers found that polymorphisms clearly existed in the 5-flanking region of *Pit-1* gene which might correlate to certain growth traits. The data suggest that these loci might serve as the potential genetic markers for goose breeds.

MATERIALS AND METHODS

Materials: Zi goose (N = 43), Wan-xi White Goose (N = 49) and Tai-hu (N = 102) 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

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

Primers design: Six primers were designed and conducted PCR according to the *Pit-1* gene sequence which researcher had cloned, 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 $U\mu L^{-1}$), 0.5 μL of forward primer (5 pmol L^{-1}), 0.5 μL of reverse primer (5 pmol L^{-1}), 1.0 μL of DNA 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_2 EDTA), 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, three 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 *Pit-1* 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:

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

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

RESULTS AND DISCUSSION

The PCR Amplification 5' sequence of pit1 gene of goose: Six primers were designed to amplify the 5' sequence of *Pit-1* gene. The results showed there was specific and clear band for each primer respectively (Fig. 1), the PCR product could be used for PCR-SSCP analysis.

Detection of the goose *Pit-1* genetic polymorphism SSCP detection 5' sequence of pit1 gene: PCR-SSCP results showed the genetic polymorphism was detected only at p4 primer. For p4 primer, there were tow alleles (named C and T) and 2 genotypes were observed (Fig. 2), defined CCand TT genotype respectively.

Sequencing analysis: DNA sequencing analysis showed there was just one mutation found (c.-930C>T) (Fig. 3), the mutation may increase or decrease MNB1a, Dof2, Dof3, PBFand Hb transcription factor binding sites.

Genotypes and allele frequency distribution in different goose group: The genotype and alleles frequency was shown in Table 2. For the P4 locus, the dominant alleles was C for three goose breeds. It was clear to see from Table 3, the distributions of the P4 locus were no difference ($p>0.05$) among the goose populations.

Least square analysis of the genotype the group and the body weight: In this study, the association of the GH gene polymorphism with the body weight was revealed

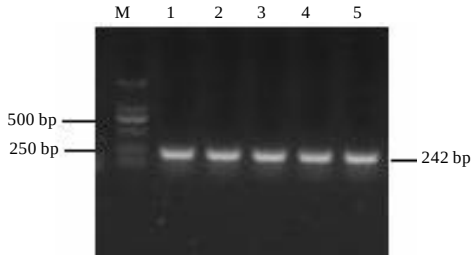

Fig. 1: The PCR products of p4 primer M Marker (DL2000)

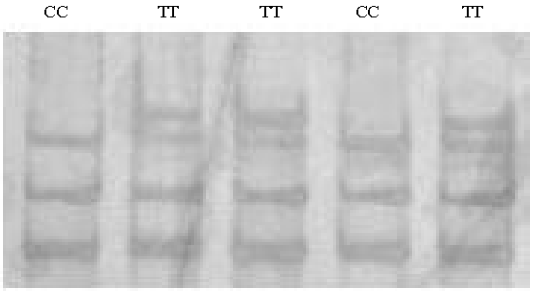

Fig. 2: SSCP analysis of PCR products of the p4 of pit1 gene

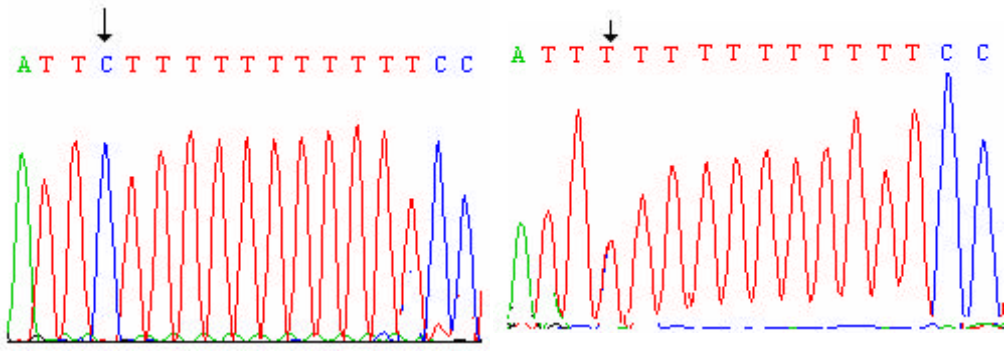

Fig. 3: Sequence of CC and TT genetypes at -930 site

Table 1: Primer pairs designed for amplification of *Pit-1* gene in goose

Primer	Sequences	Amplified length	Ann. temp
P1	F 5'- CCTGGTCGTTGTTCTTCAC -3'	129	53
	R 5'- ATAGAGCCTCCTTCCTTCC -3'		
P2	F 5' CGATAGCAAGACAAATGATG -3'	255	52
	R 5'- TGTGAAGAACAACGACCAG -3'		
P3	F 5'- GTTCCAAAGTCATTCTGTT -3'	327	47
	R 5'- AGTCATCATTTGTCTTGCT -3'		
P4	F 5'- CTTAAACCAAGAGTATCGT -3'	242	46
	R 5'- AACAGAATGACTTTGGAAC -3'		
P5	F 5'- CATTTGGTGGCGGTATCTT -3'	379	56
	R 5'- GTTGGGTCAGCCCGTAAA-3'		
P6	F 5'-GTTGGGTCAGCCCGTAAA -3'	176	56
	R 5'-ATACCGCCACCAAATGAAC -3'		

Table 2: Genotypes and allele frequency distribution in different goose groups in p4 primer

Groups	Zi goose (43)	Taihu goose (102)	Wanxi white goose (49)
CC	0.6047(26)	0.6078(62)	0.6122(30)
TT	0.3953(17)	0.3922(40)	0.3878(19)
CT	0 (0)	0 (0)	0 (0)
C	0.6047	0.6078	0.6122
T	0.3953	0.3922	0.3878

Table 3: χ^2-test of genotypes distribution in different goose groups

Primer	Groups	Wanxi white goose	Zi goose
P4	Taihu goose	0.00	0.00
	Wanxi white goose	-	0.01

$\chi^2_{0.05(1)} = 3.84$, $\chi^2_{0.01(1)} = 6.63$

Table 4: Effect of the source of variation on the value of growth trait

	F-value (Primer P4)				
Sourse	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
Group	164.908**	193.691**	150.907**	131.395**	116.560**
Genotype	0.374	2.759	9.399**	12.567**	21.385**
Group-genotype	0.304	0.315	0.290	0.183	0.754

*p<0.05, **p<0.01

(Table 4), the result showed that the group effect of the bodyweight was significantly different (p<0.01) among the group, the genotype effect of that was significantly difference (p<0.01) in 6, 8, 10 weeks. The effect of genotype-group interaction was not significantly difference (p>0.05).

Table 5: Comparison of bodyweight in different groups

Items	Zi goose	Taihu goose	Wanxi white goose
2 weeks	279.10±7.760^a	410.44±7.700^b	491.37±6.910^c
4 weeks	735.51±15.78^c	1104.00±18.25^a	1225.22±15.09^b
6 weeks	1210.80±27.06^b	1843.57±23.57^c	1905.79±30.99^a
8 weeks	1745.67±40.82^a	2379.32±29.54^b	2663.71±37.21^c
10 weeks	2090.35±39.45^c	2652.35±32.79^a	3119.29±55.44^b

The same superscripts in the same line means not significant difference (p>0.05), different superscripts means significant difference (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 difference (p>0.05) in the three goose group and the general trend was Wan-xi white goose>Taihu goose>Zi goose. That of Wan-xi white goose was the highest and greater than Taihu 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 CC>TT. In WanXi white goose and zi goose groups, there was significant difference (p<0.05) in 6 and 8 weeks, there is an extremely sigrdficant difference (p<0.01) in 10 weeks but there was no difference (p>0.05) in 6 weeks. The candidate gene approach is 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). Due to Pit-1 had played crucial regulatory function and varieties of bioactivities, Pit-1 has been regarded as a key candidate gene for production performance at present.

There are indications that variations of *Pit-1* gene are related to growth, carcass, milk productionand fatty traits in Farm Livestock (Brunsch *et al*., 2002; Song *et al*., 2005; Zhao *et al*., 2004; Jiang *et al*., 2004; Lan *et al*., 2007) however, these studies are mainly concentrated on the exons and introns of pit1 gene and few promoter polymorphism of pit1 gene researchs is reported, Recently, it has been shown that the allele A has a favorable positive effect on growth traits in different genotypes of Pit-1 5' flank region and growth performance

Table 6: Least square means standard and deviation of different genotypes in primer P4 for growth trait

Group/ weeks	Wanxi white goose		Taihu goose		Zi goose	
	CC	TT	CC	TT	CC	TT
2	491.68±10.05	490.90±08.390	410.28±09.39	410.68±13.49	285.54±10.24	267.50±11.32
4	1232.10±19.40	1214.55±24.200	1116.76±22.21	1084.53±31.48	757.21±19.76	699.76±24.47
6	1956.48±38.24*	1828.42±48.120*	1872.29±28.01	1799.05±41.02	1253.29±34.11*	1140.82±40.07*
8	2731.41±44.67*	2560.37±58.430*	2426.84±37.53*	2305.68±46.01*	1808.38±54.42*	1638.71±52.00*
10	3234.93±56.06**	2936.68±100.60**	2715.65±41.60**	2554.25±49.98**	2175.45±47.15**	1945.18±55.82**

in Sujiang pig so Promoter of Pit1 polymorphism research also has certain significance. In this study, firstly analysed polymorphisms and investigated the association between polymorphisms and growth traits in the 5' Flank of goose *Pit-1* gene, six primers are designed and only P4 primer was detected to have polymorphisms. The results showed that one SNPs were detected which were C-930T, respectively. Two kinds of genotypes (CC and TT) were detected, genotypes distribution have no significant differences ($p>0.05$) and the dominant alleles was C for three goose breeds.

The least square on genotypes and groups analysis showed that there were significant differences ($p<0.01$) on body weight of different weeks in different groups and genotype, however, there were no differences ($p>0.05$) on body weight of different weeks in genotype-group interaction effect then Comparison of bodyweight in different groups show that the average body weight of wanxi white goose was the largest, the average body weight of taihu goose was larger than zi goose.

Variance analysis on genotype show that the average body weight of CC genotype and TT genotype had significant differences ($p<0.05$) in 6, 8 weeks and had extremely significant differences ($p<0.05$) in 10 weeks. This result indicated that the goose with the CC genotype had higher body weight, Therefore this study indicated that the polymorphic site could be a genetic marker for growth trait.

CONCLUSION

This experiment was conducted to study the Polymorphism on Pit-1 5' flank region in goose. Only one SNPs was found in the sequence of Pit-1 5' flank region and 2 genotypes were detected in 3 goose populations. The least square analysis showed that the goose with the CC genotype had higher body weight, Therefore this study indicated that the polymorphic site could be a genetic marker for growth trait.

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