

## 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

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### 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- $\beta$  (TSH $\beta$ ) 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 unknown 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

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<sup>2+</sup>), 1.0 µL of dNTP mix (2.5 mM), 0.2 µL of Taq DNA polymerase (5 UµL<sup>-1</sup>), 0.5 µL of forward primer (5 pmol L<sup>-1</sup>), 0.5 µL of reverse primer (5 pmol L<sup>-1</sup>), 1.0 µL of DNA template (100 ng µL<sup>-1</sup>), 14.8 µL of ddH<sub>2</sub>O. The product of each amplification was analyzed by electrophoresis on 1% agarose gel (5 V cm<sup>-1</sup>), 1×TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM Na<sub>2</sub> EDTA), using ethidium bromide staining (1 µg mL<sup>-1</sup>). 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 CC and 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, PBF and 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

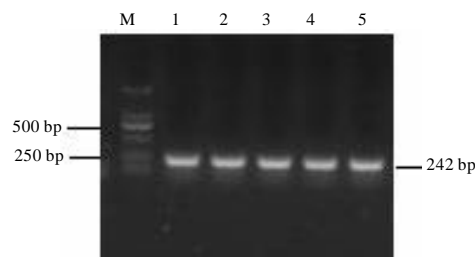


Fig. 1: The PCR products of p4 primer M Marker (DL2000)

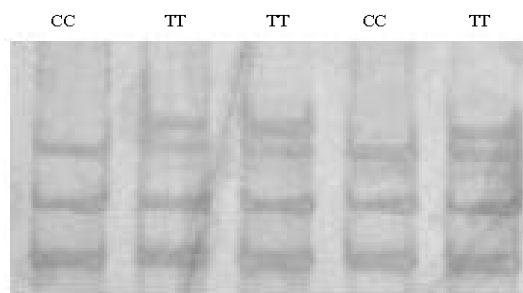


Fig. 2: SSCP analysis of PCR products of the p4 of pit1 gene

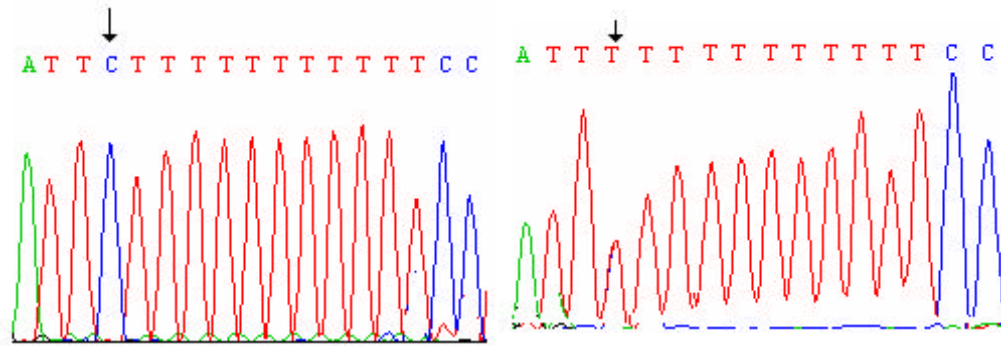


Fig. 3: Sequence of CC and TT genotypes at -930 site

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'- CTAAACCAAGAGTATCGT -3'   | 242              | 46        |
|        | R 5'- AACAGAATGACTTTGGAAC -3'  |                  |           |
| P5     | F 5'- CATTGGTGGCGGTATCTT -3'   | 379              | 56        |
|        | R 5'- GTTGGGTCAGCCCGTAAA -3'   |                  |           |
| P6     | F 5'- GTTGGGTCAGCCCGTAAA -3'   | 176              | 56        |
|        | R 5'- ATACCGCCACCAATGAAC -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:  $\chi^2$ -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

| Source         | F-value (Primer P4) |           |           |           |           |
|----------------|---------------------|-----------|-----------|-----------|-----------|
|                | 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&lt;0.05, \*\*p&lt;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 $\pm$ 7.760 <sup>a</sup>  | 410.44 $\pm$ 7.700 <sup>b</sup>  | 491.37 $\pm$ 6.910 <sup>c</sup>  |
| 4 weeks  | 735.51 $\pm$ 15.78 <sup>a</sup>  | 1104.00 $\pm$ 18.25 <sup>b</sup> | 1225.22 $\pm$ 15.09 <sup>b</sup> |
| 6 weeks  | 1210.80 $\pm$ 27.06 <sup>b</sup> | 1843.57 $\pm$ 23.57 <sup>c</sup> | 1905.79 $\pm$ 30.99 <sup>a</sup> |
| 8 weeks  | 1745.67 $\pm$ 40.82 <sup>a</sup> | 2379.32 $\pm$ 29.54 <sup>b</sup> | 2663.71 $\pm$ 37.21 <sup>c</sup> |
| 10 weeks | 2090.35 $\pm$ 39.45 <sup>c</sup> | 2652.35 $\pm$ 32.79 <sup>a</sup> | 3119.29 $\pm$ 55.44 <sup>b</sup> |

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 sigdrficant 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 production and 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/<br>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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