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Full Length Research Paper

Genetic variation of *Pit*-1 gene in Chinese indigenous and Western goose populations

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Pituitary-specific transcription factor (*Pit*-1, or GHF1, or POU1F1) is expressed in the pituitary gland; it regulates pituitary development and expression of the growth hormone, prolactin and thyrotropin β-submit genes. *Pit*-1 gene has been regarded as a candidate gene for production performance. The genetic variation of *Pit*-1 gene was investigated in five Chinese indigenous goose populations and one Western goose population by PCR-SSCP. In this study, the sequences of goose *Pit*-1 gene were identified with duck sequence; three SNPs detected were A57G in the intron, G161A and T282G were in the exon, and T282G changed the amino acid from Cys to Trp. A57G and G161A appeared only in the Western population Landoise goose. The genotypes distribution showed significant differences between different types of population.

Key words: Goose, single nucleotide polymorphisms (SNPs), pituitary-specific transcription factor gene (*Pit*-1).

INTRODUCTION

As a kind of POU (Pit-Oct-Unc)-domain binding factor, pituitary-specific transcription factor (Pit-1, or GHF1, or POU1F1) has been proven to bind and transactivate promoters of growth hormone (*GH*), prolactin (*PRL*) and thyroid-stimulating hormone-β genes (Bodner et al., 1988; Cohen et al., 1996; Miyai et al., 2005). Other bioactivities of Pit-1 have also been reported, like regulating anterior pituitary development (Li et al., 1990; Hoya et al., 1998) and pituitary cell proliferation (Castrillo et al., 1991), silencing or delaying adrenarche in human (Taha et al., 2005), being related to dwarf phenotype in mice (Camper et al., 1990), as well as including the differentiation of hepatic progenitor cells into PRL-producing cells (Lee et al., 2005). Initially activated under the control of Phophet of *Pit*-1 (PROP-1) gene, *Pit*-1 gene is auto-regulated in expression (Sornson et al., 1996) and its mRNA is present in any cell types of pituitary, whereas Pit-1 protein is mainly expressed in lactotrophs, somatotrophs and thyrotrophs, which secrete PRL, GH and TSH-β (Simmons et al., 1990).

Until now, *Pit*-1 cDNA has been identified in a variety of species, and previous studies showed that the *Pit*-1 gene comprised 6 exons in mammals, and 7 exons in birds and fishes (Tatsumi et al., 1990; Wong et al., 1992 and Yamada et al., 1993). The chicken *Pit*-1 gene cDNA was firstly been isolated and sequenced by Tanaka et al. (1999), and the chicken *Pit*-1 gene is located on chromosome 1 (GGA1), spans over 14 kb in length. The sequence of duck *Pit*-1 gene was submitted by Kansaku in 2006 (Genbank NO: AB258457), the identity of *Pit*-1 mRNA between chicken and duck was 86.35%.

Due to the crucial regulatory function and variety of bioactivities, *Pit*-1 gene has been regarded as a key candidate gene for production performance. There are indications that variations of *Pit*-1 gene are related to growth, carcass and fatty traits in pig (Yu et al., 1995; Stancekova et al., 1999; Brunsch et al., 2002; Song et al., 2005; Franco et al., 2005), growth and carcass in cattle (Zhao et al., 2004; Xue et al., 2006), and growth traits but not carcass and fatty traits in chicken (Nie et al., 2008).

In this study, the sequence of goose *Pit*-1 gene was amplified and the single nucleotide polymorphisms were analyzed in five Chinese indigenous goose populations

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Table 1. Detailed information for primer pairs used in this study.

Primer	Sequence	Position*	Product size (bp)	Annealing temp. (°C)
1	F: 5' TTGCCATGATGACTTTG 3' R: 5' CTTGAACTGATCCCTCT 3'	3123-3300	178	49.8
2	F: 5' TATCAAGCCTGCAACTC 3' R: 5' AACCACTTTCACAACCC 3'	4053-4325	263	61.0
3	F: 5'CTGGGTAGCCTTTCACATA 3' R:5'ATTTTCAGCCTTTCGTTGG 3'	4394-4798	405	54.3
4	F: 5'GAAGACACCACAGGCACA 3' R: 5' TCCAGCCACTTAGACGC 3'	6314-6579	266	49.3
5	F: 5' GCATGGTGCCCTTCTTG 3' R: 5' ATTTCAGCCCCACTCCC 3'	7928-8244	317	50.4
6	F: 5' TACTCTGACTCAACCCTT 3' R: 5' ACTCGTGATGCTCCTT 3'	10767-11061	295	60.7

The position was calculated according to duck *Pit*-1 gene sequence.

and one Western goose population. The results may help to understand the genetic effect of *Pit*-1 gene on goose productive traits.

MATERIALS AND METHODS

Goose populations

Five Chinese indigenous goose populations, Wanxi white, Shitou, Zi, Siji, Zhedong white and one Western population Landoise were selected. The total number of samples was 333 individuals; all the individuals were raised under the same environment and management.

Primers design

Six pairs of primers were designed according to the DNA sequence of duck *Pit*-1 gene (Genbank NO: AB 258457). These primers were used to amplify 6 exons of goose *Pit*-1 gene (Table 1).

SNPs identification with PCR-SSCP technique and sequencing confirmation

Goose genomic DNA was extracted from blood sample, and diluted to 100 ng/μL. PCR was performed in 20 μL mixture containing 100 ng of goose genomic DNA, 10×PCR buffer 2 μL, $MgCl_2$ 1.8 μL, 10 pmol/μL primers 1 μL, 25 μM of each dNTP 1.5 μL, 1.0 U *Taq* DNA polymerase (TakaRa Biotechnology Dalian Co., Ltd.) and 11.5 μL ddH_2O. PCR was run with the following procedure: 95°C for 10 min, followed by 38 cycles of 1 min at 94°C, 1 min at annealing temperature (49.8 - 61°C), 1 min at 72°C, and a final extension of 10 min at 72°C. Genotypes of all the primers were observed by PCR-SSCP procedure as follows: 10 μL PCR product was mixed with 5 μL loading buffer, heating at 98 °C for 10 min, then bathing in ice for 5 min and visualizing with 10% polyacryamide gel electrophoresis. The PCR fragments were purified with a DNA fragment purification kit (TakaRa Biotechnology Dalian Co., Ltd.), then cloned and sequenced in the company (Sangon Biological Engineering Technology Company, Shanghai, China).

Statistical analysis

Chi-squares analysis was done by *Chi*-square calculator V1.51; the sequences alignment was carried out by DNA man 5.22 software.

RESULTS

The sequence confirmation of goose *Pit*-1 gene fragments

Three individuals of each primer/genotype were selected randomly to clone and sequence. The results were shown in Figures 1 and 2. And the identity of sequences amplified by each primer between goose and duck was 94.41, 91.21, 94.07-94.32, 92.91, 92.77 and 96.32%, respectively, from primer 1 to 6.

The genetic variation of goose *Pit*-1 gene fragment

Among the 6 primers used, only 3 SNPs were detected by primer 3. The mutations were A57G, G161A and T282G, respectively (Figure 3) (the number was calculated from the first base of amplified fragment by primer 3). The A57G mutation was *in intron*, and the other two were *in exon*. The G161A was synonymous mutation, and T282G changed the amino acid from Cys to Trp. Mutations A57G and G161A only appeared in Landoise goose.

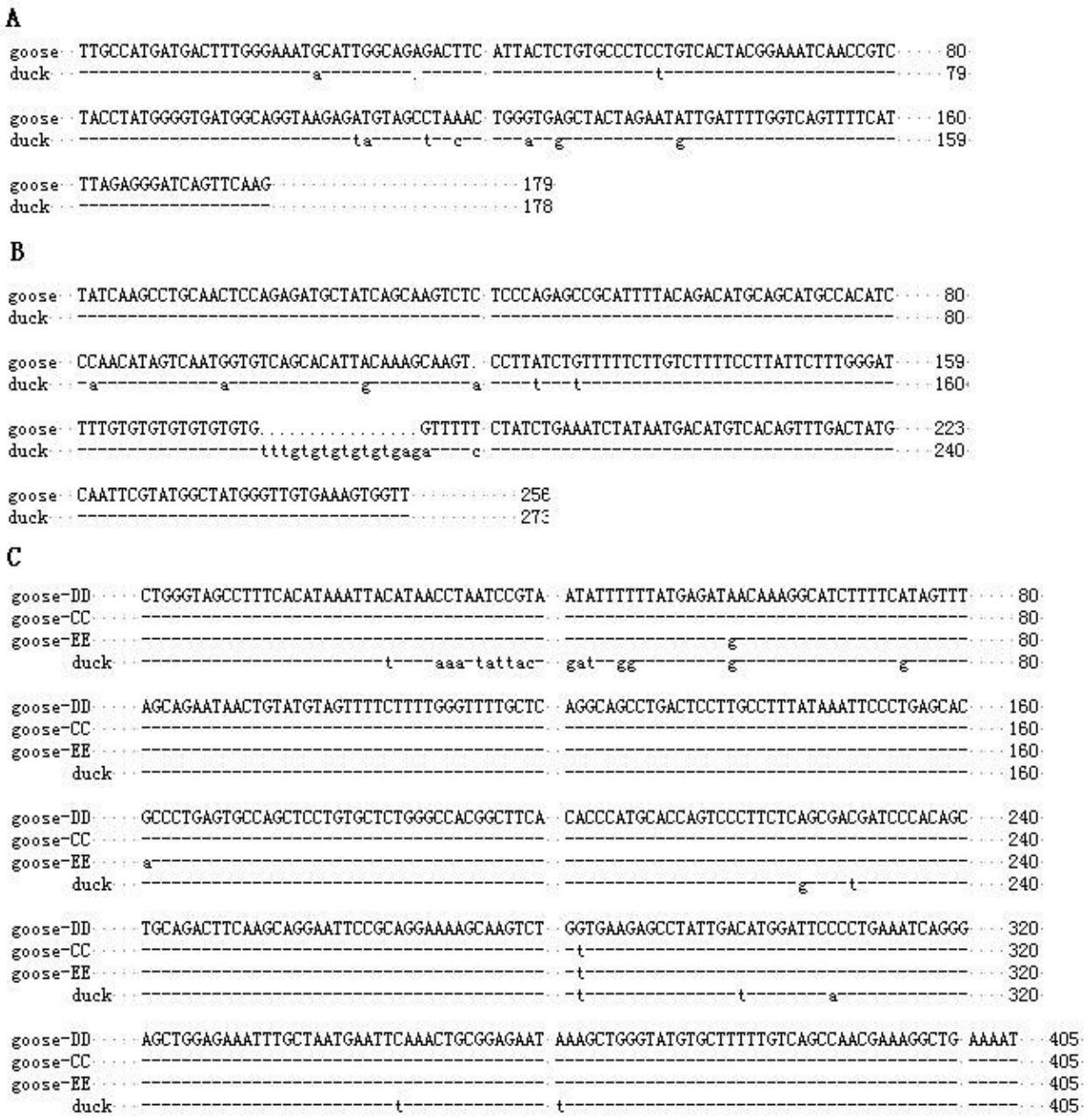

Figure 1. The sequences of primer 1-3 and the alignment with duck, "-" meansthe identical base. A, Sequence amplified by primer 1; B, Sequence amplified by primer 2; C, Sequenced amplified by primer 3; goose-DD, goose-CC and goose-EE represent different homozygotes.

Four genotypes CC, CD, DD and EE were generated among 6 populations (Figure 4); the genotypes distribution and gene frequency of the polymorphic primer in 6 populations were presented in Table 2. Compared with 5 Chinese populations, only EE genotype was appeared in Landoise goose, and this genotype was not found in the other five populations, which was corresponded to the mutation loci. The distribution of genotypes showed significant difference between different types of population (Table 3).

DISCUSSION

Six *exons* in goose *Pit*-1 gene were amplified according to duck *Pit*-1 gene sequences. The results suggested that the sequence of *Pit*-1 gene was highly conserved between goose and duck. Sequences of goose *Pit*-1 gene amplified in this study were identical to the goose *Pit*-1 gene partial sequences in Genbank (EF 457938, EF 43635).

Jiang et al. (2004) reported that the A980T mutation of

Figure 2. The sequences of primer 4-6 and the alignment with duck, "-" means the identical base. D, Sequence amplified by primer 4; E, Sequence amplified by primer 5; F, Sequenced amplified by primer 6.

chicken *Pit*-1 gene cDNA was significantly correlated with 8 weeks' bodyweight. Nie et al. (2005) detected 23 SNPs in chicken *Pit*-1 gene with HPLC technology, 3 were in 3' UTR regions, 16 were in *introns*, and there was a 57 bp deletion/insertion in intron 2. The 57 bp deletion/insertion in *intron* 2 significantly affected the body weight, breast muscle weight and leg muscle weight of 1-8 weeks' age in chicken (Qiu et al., 2006). Nie et al. (2008) investigated the correlation between the productive performance and 5 SNPs detected preciously (Nie et al., 2005), and found that the polymorphisms of *Pit*-1 gene and their haplotypes were significantly associated with chicken growth traits. Our previous study suggested that, two deletion/insertion mutations in *intron* of *Pit*-1 gene exerted significant effects on early bodyweight of goose (Cheng et al., 2008).

In this study, three SNPs were detected with the method of PCR-SSCP and sequencing. Considering the essential effects of *Pit*-1 gene on animal traits that previous studies have proved, it can be presumed that the mutations detected in this study may affect some production traits of goose.

Among the six populations, Landoise goose was a synthetic line introduced from France. It was bred with two Western breeds and mainly used for Foie Gras commercially. Shitou goose, Zhedong white goose and Siji goose are meat-type goose, Wanxi white goose is a feather-meat dual type goose, and Zi goose is a small egg-type goose. All the individuals of Landoise goose appeared with EE genotype, and EE genotype was absent in 5 Chinese indigenous geese; the proportion of DD genotype was dominant in Shitou goose, Siji goose and Zhedong white goose, which was significantly different from Wanxi white goose and Zi goose. This result suggested that goose *Pit*-1 gene may affect the traits of different populations in different ways.

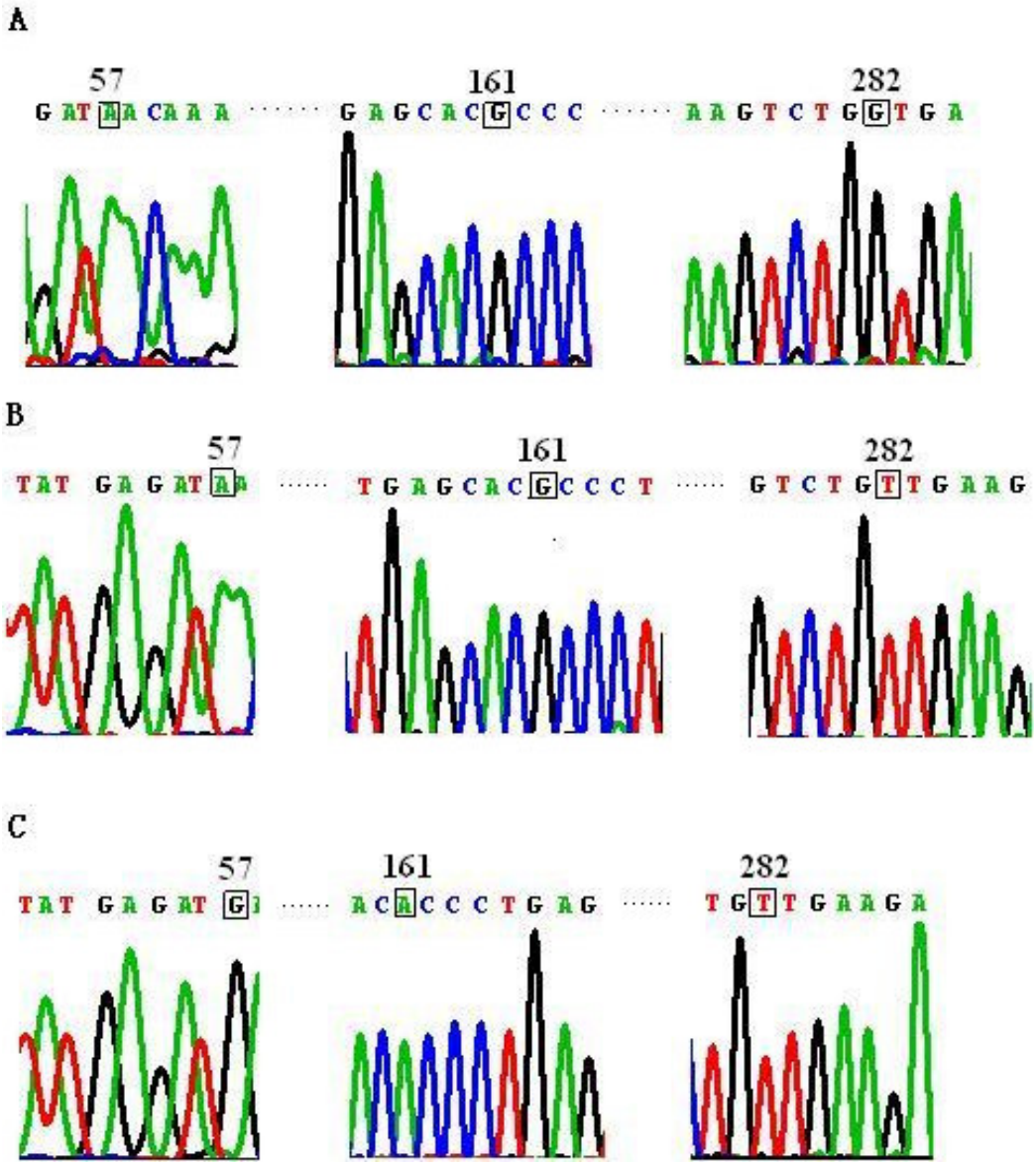

Figure 3. Chromatograms showing sequence variations at each position within the sequence of *Pit*-1 gene detected by primer 3, bases in frame were the mutation positions. A, DD genotype; B, CC genotype; C, EE genotype.

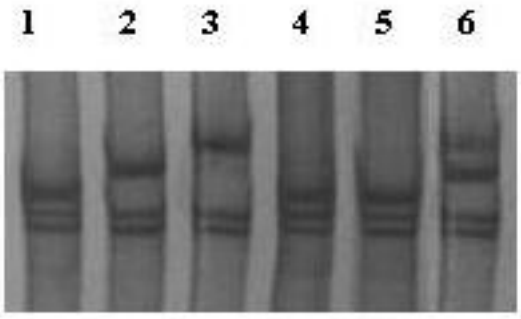

Figure 4. Different genotypes visualized by electrophoresis. 1, 4, 5, EE genotype; 2, DD genotype; 3, CC genotype; 6, CD genotype.

The absence of other three genotypes in Landoise goose may be due to the different origins. Chinese indigenous geese originated from Swan goose (Anser cygnoides) and Landoise goose originated from Graylag (Anser anser) (Chen and Wang, 2005). But also, it could be the result of long-term selection and breeding for Foie Gras traits in goose. EE genotype and DD genotype may be beneficial to Foie Gras traits and muscle growth, respectively; both of them can be used as a genetic marker in marker-assisted selection. And further studies should be conducted in cross population of Chinese indigenous goose and Landoise goose to explore the accurate effects of alleles D and E on goose traits.

Table 2. Sample size, genotype and gene frequency in 6 goose populations for the polymorphic primer pairs.

Population	Sample size	Genotype frequency				Allele frequency		
		CC	CD	DD	EE	C	D	E
Shitou goose	67	1(0.0149)	17(0.2537)	49(0.7313)		0.1418	0.8582	0.0000
Wanxi white goose	70	17(0.2428)	29(0.4143)	24(0.3429)		0.4500	0.5500	0.0000
Zi goose	55	14(0.2545)	24(0.4364)	17(0.3091)		0.4727	0.5273	0.0000
Siji goose	43	4(0.0930)	11(0.2558)	28(0.6512)		0.2209	0.7791	0.0000
Zhedong white goose	63	2(0.0317)	11(0.1746)	50(0.7937)		0.1190	0.8810	0.0000
Landoise goose	34	0(0.000)	0(0.000)	0(0.000)	34(1.0000)	0.0000	0.0000	1.0000

Table 3. *Chi*-Square analysis of genotype distribution of *Pit-1* between different geese.

χ^2	Wanxi white goose	Zi goose	Siji goose	Zhedong white goose	Landoise goose
Shitou goose	25.86**	27.06**	3.76	1.21	101**
Wanxi white goose		0.16	10.61**	28.79**	104**
Zi goose			11.78**	29.68**	89**
Siji goose				3.21	77**
Zhedong white goose					97**

**Means the extreme significant difference ($P < 0.01$).

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