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Analysis of Two *Pit-1* Gene Polymorphisms and Relationships with Growth Performance Traits in Mink

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Abstract: The pit-1 has been implicated in regulation of body weight. Its gene sequence and protein function exhibit high evolutionary conservation and have been studied extensively in humans, mice, pigs and chickens. Mink pit-1 is nearly identical to dog pit-1 but little is know about its function. Thus, researchers investigated whether polymorphisms in th pit-1 exon4 and exon6 were associated with mink growth traits. Five population of mink were assessed for growth traits for use in statistical correlation analysis with genomic sequence data. Two SNPs were detected in the pit-1 exon4 and exon6 of each population: 43C>T and 143 A>G. The 43C>T mutation produced three genotypes: AA, BB and AB. Statistical analysis of variance revealed that 43C>T polymorphism was associated with Body Weight (BW) and Carcass Weight (CW) in black mink; the 143A>G mutation produced three genotypes as well: CC, DD and CD; statistcal analysis of variance revealed that the 134A>G polymorphism was associated Body Weight (BW) in black mink ($p<0.05$). The interaction of 43C>T and 143A>G was discussed through combination genotype analysis. The result showed that the combined genotype had significant association with Body Lenght (BL) in sapphire mink and Body Weight (BW) and Carcass Weigth (CW) in black mink ($p<0.05$). The findings suggest that the *pit-1* gene could be a qualitative trait locus or linked to a major gene that affects growth traits in mink.

Key words: Mink, *pit-1* gene, polymorphism, combination genotype, growth traits

INTRODUCTION

Pit-1 (also named POUIF1 or GHF-1) is a member of the POU homeodomain family of transcription factors (Bodner *et al*., 1988; Ingraham *et al*., 1988) which regulates pituitary development and the expression of the growth hormone, prolactin and thyrotropin β-subunit genes (Cohen *et al*., 1996). Until now, Pit-1 encoding cDNAs were cloned in 18 species from three different Classes: Mammalia, Aves and Actinopterygii as summarized by Bastos *et al*. (2006). The human *Pit-1* gene is located on chromosome 3 p11, the genomic sequence of this gene is 44.58 kb in size. The mRNA of Ccdl, 4803 bp in length and it has 6 exons which encodes a protein of 472 amino acids (Ohta *et al*., 1992). Association studies have shown that Pit-1 is related to many production traits in domestic animals. HinfI polymorphism has been reported of the Bull *pit-1* gene which revealed two alleles A and B (Renaville *et al*., 1997), the A allele was found to be superior for milk, protein yields and body depth; A *Pit-1* gene mutations in humans have been reported as causing Combined Pituitary Hormone Deficiency (CPHD) with low or zero levels of *TSH*, growth hormone and prolactin (Cohen and Radovick, 2002). Genetic variations of *Pit-1* gene are associated with body weight at 8 weeks of age indicating that the SNP in *Pit-1* gene is a potential molecular marker for early growth rate in chicken (Jiang *et al*., 2004); Likewise, Qun *et al*. (2006) found that a 57 bp mutation in the intron2 of chicken *pit-1* gene was significantly associated with body weight ($p<0.05$), A PstI polymorphism at 3'UTR of goat *Pit-1* gene was found by Lan *et al*. (2009), TT genotype was associated with superior cashmere yields in 2, 4 and 5 years old individuals as well as with average cashmere yield. These researchers considered that this SNP in *Pit-1* gene is a potential molecular marker.

Little is known about the mink *pit-1* gene, its biological function or nucleotide mutations that affect such. Taking into consideration the well-characterized features of pit-1 in other animals, it is reasonable to propose pit-1 involvement in the growth traits of minks. Therefore, researchers investigated the *pit-1* gene sequences in five populations of mink to identify any Single Nucleotide Polymorphisms (SNPs) present in

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exon4 and exon6 to determine whether those SNPs were associated with desirable growth traits. Researchers expect the findings to provide novel insights into the mink *pit-1* gene that will benefit future studies into this and other mink genotypes and support the development and application of molecular breeding.

MATERIALS AND METHODS

Experimental animals and phenotypic measurements: Genomic DNA samples were obtained from 240 individuals belonging to five populations: standard-pitchy mink (121), sapphire mink (45), coffee mink (56), white mink (57) and black mink (60). All five populations were reared in the provinces of Liaoning Jinzhou mink breeding. Growth trait measurements included Body Weight (BW), Body Length (BL), Body Height (BH), Chest Circumference (CC), Abdomen Circumference (AC) and Carcass Weight (CW).

DNA preparation, primer design and PCR amplification: DNA samples were extracted from muscle were obtained from all minks which was extracted using standard methods. Isolated DNA from each mink was individually dissolved in sterile water to obtain a concentration of 50 ng μL^{-1} and stored at -20°C. The PCR primers were designed according to the dog *pit-1* gene sequence deposited in GenBank (Accession No. NM001006949.1). Three pairs of primers were designed and the sequences and size of the amplified fragments are shown in Table 1. PCR reaction mixtures (25 μL) included 50 ng of genomic DNA, 25 pmol of each primer, 2.5 μL 10×PCR buffer, 2 μL dNTP and 1.5 U Taq DNA polymerase. Thermal cycling conditions were showed in Table 2.

Table 1: The primers of *PIT-1* gene

Primers sequence (5'→3')	Amplification region	Amplification length (bp)
Pit-1-1F 5' CCCTTTACTTCGGCTGACA 3'	exon1	117
Pit-1-1R 5' GTTGGTGGCGTGGTTGGA 3'		
Pit-1-4F: 5' GATACACCCAAACCAACG 3'	exon4	150
Pit-1-4R: 5' CTTCCTCCAGCCATTTAG 3'		
Pit-1-6F: 5' TCAGACTTGTTTTCACCC 3'	exon6	164
Pit-1-6R: 5' TATTGCTGCTAAGGATGC 3'		

PCR-Single Strand Conformation Polymorphism (SSCP) analysis and DNA sequencing: The PCR product was diluted 1:5 with loading buffer (98% formamide, 10 mmol L^{-1} EDTA, pH 8.0, 0.025% xylene cyanol FF, 0.025% bromophenol blue and 2% glycerol).

After denaturation by incubation at 98°C for 10 min, the mixture was immediately chilled on ice for 10 min and then loaded onto a 16% acrylamide/bisacrylamide (arc: bis, 29:1) gel.

Bands were resolved by electrophoresis at 10 V cm^{-1} for 16 h and detected by using the Standard Silver Staining Method. For each polymorphism, three PCR products were amplified and purified. Sequencing was carried out by the ABI377 sequencer.

Statistical analysis: To determine associations between the SNPs and carcass traits, the PROC-GLM procedure in the SAS Statistical Software (Version 8.2) was used. The linear model was as follows:

$$Y_i = \mu + G_i + e$$

Where:

Y = Growth traits
μ = Mean number of population
G_i = The effect of genotype
e = The random error

The analysis model of combined genotype was as followed:

$$Y = \mu + G(43C<T + 143A<G) + e$$

Where:

Y = The observed values of traits
G = The genotype effect
μ = The mean of the population
G(43C>T and 143A>G) = The combination genotype effect
e = The random error

The χ^2-test was used to determine significant differences in allele frequencies. A $p<0.05$ was considered statistically significant.

Table 2: Parameters of PCR amplification

Primers	Initial denaturation	Denaturation	Annealin	Elongation	Cycle	Elongation
Pit-1-1	94°C, 7 m	94°C, 0 sec	55.5°C, 10 sec	72°C, 4 sec	36	72°C, 5 m
Pit-1-4	94°C, 5 m	94°C, 30 sec	56.0°C, 35 sec	72°C, 30 sec	35	72°C, 8 m
Pit-1-6	94°C, 5 m	94°C ,10 sec	48.8°C, 10 sec	72°C, 13 sec	40	72°C, 7 m

RESULTS AND DISCUSSION

Polymorphisms in the exon4 and exon6 of mink *pit-1* gene: The PCR product was 117 bp in length in exon1 of mink *pit-1* gene (Fig. 1), there no polymorphism was found in exon1.

The PCR products was 150 bp in length in exon4 of mink *pit-1* gene (Fig. 2), three unique SSCP genotypes were observed (Fig. 3). The SNP accordingly contributed to AA, AB and BB genotypes and the frequencies of A and B of five populations were shown in Table 3. In order to confirm this polymorphism, the PCR product of the polymorphic animal was sequenced. This polymorphism, consisting of a C fi T transition at codon 43, leads to a change from threonine to methionine (Fig. 4).

The PCR products was 164 bp in length in exon6 of mink *pit-1* gene (Fig. 5)the exon 6 of mink *pit-1* gene, three unique SSCP genotypes were identified. The frequencies of C and D of five populations were showed in Table 4, the PCR product of the polymorphic animal was also sequenced. This polymorphism, consisting of a A fi G transition at codon 143, leads to a change from lysine to arginine.

Association of *PIT-1* gene SNPs with growth traits: *Pit-1* gene polymorphisms in the exon4 of each population correlated to growth traits. The 43C>T polymorphism had some influences with chest circumference ($p = 0.170$) of standard-pitchy mink, chest circumference ($p = 0.123$) and carcass weight ($p = 0.068$) of coffee mink, body weight ($p = 0.196$) of white mink, body length ($p = 0.152$), chest circumference ($p = 0.094$) and abdomen circumference ($p = 0.142$) of black mink ($p<0.2$) and was significantly associated with carcass weight ($p = 0.010$) of black mink, body length ($p = 0.004$) of sapphire mink, body length ($p = 0.003$) of black mink ($p<0.05$), Furthermore, coffee mink with the AB and AA genotypes had significantly

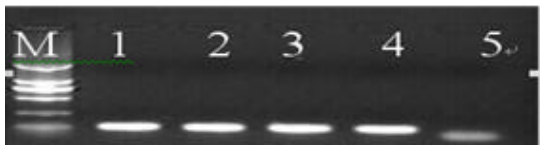

Fig. 1: PCR amplification results of primer 1 (primer 1) 1-4: PCR products; 5: control; M: DL 2000

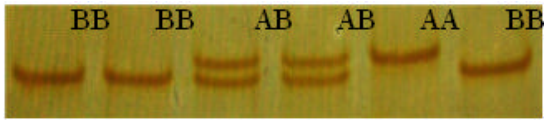

Fig. 2: PCR-SSCP results of different genotype

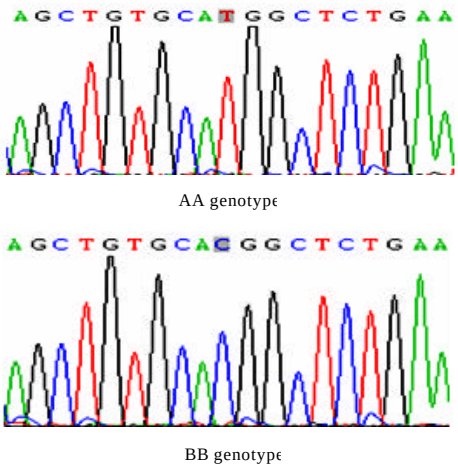

Fig. 3: Sequencing results of mutational site of exon4 of *PIT-1* gene

Table 3: Genotypes and allele frequencies of the mutation 43C<T of *PIT-1* gene

		Genotype frequency			Allele frequency	
Population	No.	AA	BB	AB	A	B
Standard-pitchy	121	0.298 (36)	0.140 (17)	0.562 (68)	0.579	0.421
Coffee	56	0.393 (22)	0.054 (3)	0.553 (31)	0.670	0.330
Sapphire	45	0.356 (16)	0.067 (3)	0.578 (26)	0.644	0.356
White	57	0.088 (5)	0.719 (41)	0.193 (11)	0.184	0.816
Black	60	0.150 (9)	0.317 (19)	0.533 (32)	0.417	0.583

Table 4: Genotypes and allele frequencies of the mutation 143A<G of *PIT-1* gene

		Genotype frequency			Allele frequency	
Population	No.	CC	DD	CD	C	D
Standard-pitchy	115	0.270 (31)	0.139 (16)	0.591 (68)	0.565	0.435
Coffee	58	0.241 (14)	0.345 (20)	0.414 (24)	0.448	0.552
Sapphire	46	0.087 (4)	0.391 (18)	0.522 (24)	0.348	0.652
White	56	0.821 (46)	0.018 (1)	0.161 (9)	0.902	0.098
Black	54	0.685 (37)	0	0.315 (17)	0.843	0.157

Table 5: Effects of 43C<T mutation on CC and CW in sapphire mink

Genotype	No.	CC (g)	CW (g)
AA	22	22.250±0.375^{a}	0.889±0.022^{ab}
AB	31	22.371±0.312^{a}	0.929±0.019^{a}
BB	3	20.167±1.092^{b}	0.788±0.057^{b}

Table 6: Effects of 43C<T mutation on BL in coffee mink

Genotype	No.	BL (cm)
AA	16	36.437±0.288^{b}
AB	26	36.384±0.201^{b}
BB	3	38.666±0.333^{a}

Table 7: Effects of 43C<T mutation on BW and CW in black mink

Genotype	No.	BW (g)	CW (g)
AA	9	1.189±0.037^{a}	0.769±0.016^{a}
AB	32	1.047±0.032^{b}	0.706±0.017^{b}
BB	19	1.209±0.037^{a}	0.778±0.017^{a}

Table 8: Effects of 143A<G mutation on BL in sapphire mink

Genotype	No.	BL (cm)
CC	4	37.750±0.947^{a}
CD	24	36.375±0.215^{b}
DD	18	36.388±0.244^{b}

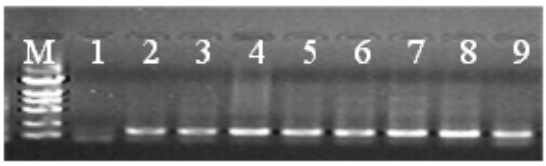

Fig. 4: PCR amplification results of primer 3 (Primer 3) 2-9: PCR products; 1: control; M: DL 2000

Fig. 5: PCR-SSCP results of different genotype

higher CC than the BB genotype and coffee mink with AB genotype had significantly higher CW as to minks harboring AB or AA genotypes (Table 5). For the sapphire mink, the BB genotype was associated with significantly higher BL as compared to minks harboring the AA and AB genotypes (Table 6). Moreover, the black mink with BB and AA genotypes were associated with significantly higher BW, CW than in minks with AB (Table 7).

The 143A>G polymorphism had some influences with body length (p = 0.079) of sapphire mink, abdomen circumference (p = 0.187) of white mink, body weight (p = 0.183) and carcass weight (p = 0.127) of black mink (p<0.2), the polymorphism was significantly associated with body length (p = 0.032) of black mink (p<0.05). Furthermore, sapphire mink with the CC genotype had significantly higher BL than the CD or DD genotypes (Table 8). In black mink, only two genotypes were

Table 9: Effects of 143A<G mutation on BL in black mink

Genotype	No.	BL (cm)
CC	37	31.878±0.269^{b}
CD	17	32.941±0.406^{a}

Table 10: Multiple comparison of different genotype combination in sapphire mink

Genotype	No.	AC (g)
AACC	4	27.125±1.434^{ab}
AACD	16	28.219±0.664^{a}
AADD	14	29.286±0.784^{a}
ABCC	16	28.906±0.713^{a}
ABCD	47	27.915±0.321^{ab}
ABDD	2	25.000±0.500^{b}
BBCC	11	27.409±0.642^{ab}
BBCD	5	27.000±1.140^{ab}

Table 11: Multiple comparison of different genotype combination in genotype combination in standard-pitchy mink

Genotype	No.	BL (cm)	BH (cm)
AACD	3	36.667±0.667^{bc}	12.667±0.667^{ab}
AADD	13	36.385±0.331^{bc}	13.077±0.383^{a}
ABCC	2	35.000±0.000^{c}	13.000±0.000^{a}
ABCD	21	36.333±0.232^{bc}	13.548±0.223^{a}
ABDD	2	37.000±0.000^{b}	11.000±0.000^{b}
BBCC	3	38.667±0.333^{a}	14.333±0.882^{a}

detected and the CD genotype had significantly higher BL as to minks harboring CC genotypes (Table 9).

Association of genotype combination with growth traits: The genotype combination had some influences with abdomen circumference (p = 0.162) of standard-pitchy mink, body height (p = 0.173) of sapphire mink, body length (p = 0.086) and body height (p = 0.078) of black mink (p<0.2). While the polymorphism was significantly associated with body length (p = 0.026) of sapphire mink, carcass weight (p = 0.012) and body weight (p = 0.003) of black mink (p<0.05). Furthermore, Standard-pitchy mink with AADD genotype had the highest AC with other genotype and had significantly higher AC than the ABDD genotype (Table 10). Sapphire mink with the BBCC genotype had significantly higher BL than the other five genotypes and the BBCC genotype had the highest BH which was associated with significantly higher BH as compared to minks harboring the ABDD genotype (Table 11) for the black mink, the AACD genotype was associated with significantly higher BW as compared to minks harboring the ABCC genotype, the BBCD genotype had significantly higher BH than the AACD and ABCC genotypes while the black mink with BBCD had significantly higher CW than the other five genotypes (Table 12).

Several studies identified polymorphisms of the *Pit-1* gene and their associations with quantitative traits (Moody *et al*., 1996; Brunsch *et al*., 2002). In this study, we detect the polymorphism of the *Pit-1* gene in five mink populations by PCR-SSCP and DNA sequencing methods

Table 12: Multiple comparison of different genotype combination in black mink

Genotype	No.	BW (g)	BH (cm)	CW (g)
AACC	3	1.117±0.011^{ab}	14.333±0.667^{ab}	0.725±0.020^{bc}
AACD	5	1.257±0.048^{a}	13.000±0.000^{b}	0.804±0.011^{b}
ABCC	17	0.099±0.034^{b}	13.029±0.279^{b}	0.684±0.021^{c}
ABCD	11	1.143±0.066^{ab}	13.727±0.407^{ab}	0.742±0.033^{bc}
BBCC	17	1.221±0.040^{a}	13.853±0.209^{ab}	0.772±0.018^{bc}
BBCD	2	1.180±0.000^{ab}	15.000±0.000^{a}	0.900±0.000^{a}

to determine its contributions to growth traits. Researchers began the search for Pit-1 polymorphisms by using the SSCP technique which is considered a useful tool for preliminary DNA polymorphism studies. Sensitivity of this technique is negatively correlated with the length of the analyzed DNA fragment (Skorczyk *et al*., 2007); therefore, researchers devised three primers and generate fragments of sufficient length (<250 bp) for detecting polymorphisms. By this approach, researchers only detected two polymorphisms within the exon4 and exon6 of mink *Pit-1* gene that may significantly affect the growth traits associated with Pit-1 protein function. The results of least square analysis confirmed that a significant association existed between the AB genotype of coffee mink and abdomen circumference, carcass weight and between the BB genotype of sapphire mink and body length and between the BB genotype of black mink and body weight, carcass weight and between the CC genotype of sapphire mink and body length and between the CD genotype of black mink and body length while the exact mechanism underlying the growth trait associated polymorphisms detected in the study remains unclear.

According to the study, the polymorphisms of *pit-1* gene have significant effect on the body weight, body length and carcass weight. Renaville *et al.* (1997) found a positive association of allele A of the POU1 F1 HinfI polymorphism with milk and protein yields in Holstein-Friesian dairy cattle. Interestingly, Zwierzchowski found no relationship of this marker with growth and carcass traits in beef cattle, the similar result was showed in Angus beef cattle (Zhao *et al*., 2004) which found no significant associations were observed between polymorphisms of *pit-1* gene in Angus beef cattle and growth and carcass traits. Nie *et al.* (2008) found that polymorphisms of *Pit-1* gene and their haplotypes were associated with chicken growth traits but not with carcass and fatty traits. However, Pan *et al.* (2008) also found no significant associations of the TaqI polymorphism of *pit-1* gene with body weight and average daily gain for different growth periods (6, 12, 18 and 24 months old) were observed ($p<0.05$) as well as for body sizes ($p<0.05$). Therefore, this genetic marker seems to have different effects in different populations and species.

The relationship between candidate genes of polymorphic loci and traits can take advantage of haplotype analysis or genotype combination analysis. The research of the correlation between the human disease and gene showed that the linkage analysis of a few loci and traits was more accurate than the single loci (Schafer and Hawkins, 1998). The genotype combination was not a simple sum of the effect of each gene but slightly higher than the best single genotype effect (Chen *et al*., 2000). Haplotype analysis was used in chicken *H-FABP* gene by PCR-SSCP, the result showed that *H-FABP* gene haploid AB-AB-BB-BB-AA and BB-AB-AB-BB-BB with a higher Intramuscular Fat Content (IMF), the maximum effect value and the IMF which had significantly differences with the other genotypes was the best genotype combination (Wang, 2006). The combination genotype analysis was used in the research of the interaction of MC3R-T91G and MC4R-A903G in pigeon, the result showed that the combined genotype had significant association with holo-carcass weight ($p<0.05$). BBAA genotype birds had a higher holo-carcass weight than AABB genotype birds and BBAA genotype was the beneficial genotype for the growth of body weight (Li *et al*., 2008).

In this study, the interaction of 43C<T mutation and 143A<G mutation in *PIT-1* gene was discussed through combination genotype analysis. The result showed that the interactive effect of two SNPs was both positive and negative and irregularity. This is consistent with earlier study of the combination genotype analysis of five genes (*ESR*, *FSHβ*, *PRL*, *PRLR* and *NCOA1*) and little size in pigs (Shi *et al*., 2006). According to the study, 43C<T polymorphism had some influence in abdomen circumference of standard-pitchy mink ($p<0.2$) but there was no significant difference between different genotype and the 143A<G polymorphism had no influence in growth traits of standard-pitchy mink while the combination genotype analysis found that there was significantly association between the genotype and abdomen circumference which may be due to the positive effect of combination genotype. While in the study of coffee mink and white mink researchers found that both the 43C<T and 143A<G polymorphism has some influence in some growth traits ($p<0.2$) but the result of combination genotype showed no correlation between the genotype and traits which may be the negative effect of combination genotype. In Sapphire mink, the result of combination genotype was consistent with single soci and the BBCC genotype had significantly higher BL than the BB genotype and CC genotype, the BBCC genotype was the preponderant genotype in body length of

sapphire mink. The similar result was found in black mink, BBCD genotype was the preponderant genotype in body weight of black mink.

CONCLUSION

The study demonstrated that polymorphisms in exon4 and exon6 of mink *pit-1* gene contribute to variance of growth traits in different populations. Thus, the *pit-1* gene may be an important target of molecular breeding to mink.

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