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Molecular Cloning and Polymorphism Analysis on Exon 2 of Thyroid Hormone Responsive Spot 14 Gene in Geese

Na Qiao, Rong-Xue Zhao, Xue-Bin Wang, Qi Xu, Jin-Hua Cheng,
Wen-Ming Zhao and Guo-Hong Chen
College of Animal Science and Technology, Yangzhou University,
Yangzhou 225009, China

Abstract: This experiment was conducted to study the Polymorphism on exon 2 of THRSPα gene in goose. The Single Nucleotide Polymorphism (SNP) of (Thyroid Hormone Responsive Spot 14 gene) THRSPα gene was investigated in Zi geese, Wanxi White geese, Zhedong White geese and Shitou geese. One primer for exon 2 of THRSPα gene were designed according to the homologous sequence of duck. Six SNPs were found in the sequence of THRSPα gene and 6 genotypes AA, BB, CC, AB, AC and BC were detected in 4 goose populations. All the populations were in Hardy-Weinberg equilibrium at this polymorphic site ($p>0.05$). The results confirmed that there were polymorphisms in the exon 2 of THRSPα gene.

Key words: Goose, Thyroid Hormone Responsive Spot 14 (THRSPα) gene, Single Nucleotide Polymorphism (SNP), polymorphism, genetic variation, China

INTRODUCTION

The Thyroid Hormone Responsive Spot 14(THRSPα) gene also known as Spot 14 began nearly 3 decades ago. Investigators used two dimensional gel electrophoresis of *in vitro* translated products to survey the effect of thyroid hormone on rat hepatic gene expression (Seelig *et al*., 1981). It encodes a small acidic protein expressed predominately in the adipogenic tissues such as the lactating mammary gland, fat and the liver (Jump *et al*., 1984; Jump and Oppenheimer, 1985). Among the transcripts that responded to both thyroid hormone and a lipogenic (high carbohydrate) diet was the mRNA for Spot 14 so named because it was the 14th spot on the gel noted to change in response to thyroid hormone status.

The chicken THRSP gene was found in livers of chickens divergently selected for fast or slow growth rates. It was cloned by in silico EST assembling and was identified to duplicate into 2 paralogs, THRSPα and THRSPβ (Wang *et al*., 2004). Sequence analysis of mammal and chicken THRSP genes demonstrated that both of them shared a similar gene organization with 2 exons and an intron (Grillasca *et al*., 1997; Wang *et al*., 2004). Duck THRSPα and THRSPβ were predicted to encode peptides with 133 amino acids. A high percentage (73.1%) of G and C nucleotides were found in the 3′ untranslated region of duck THRSPβ cDNA (Zhan *et al*., 2006). Geese and ducks belong to waterfowl and both of them share de novo fatty acid biosynthesis mainly in the liver. THRSP gene may play an important role in avian adipogenesis and it is necessary to get DNA sequence of goose THRSP gene for future functional genomic investigations. In this study, the sequence of goose THRSPα gene was amplified and the single nucleotide polymorphisms were analyzed in four Chinese indigenous goose populations. The results may help to understand the genetic effect of THRSPα gene on goose productive traits.

MATERIALS AND METHODS

Goose populations: Four Chinese indigenous goose populations including 59 Zi geese from Jilin, 66 Wanxi white geese from breeding station in Anhui Province, 71 Zhedong white geese provided by National Waterfowl Germplasm Resource Pool in Jiangsu province, 67 Shitou geese from breeding station in Guangdong province, China. The total number of samples were 263 individuals, all the individuals were raised under the same standard condition.

Primers design: One pairs of primer were designed according to the DNA sequence of duck THRSPα gene (Genbank NO: DQ334339). This primer was used to amplify exon 2 of goose THRSPα gene (Table 1).

Table 1: Primer pairs designed for amplification of THRSPα gene in goose

Primer	Sequence (5′-3′)	Position*	Product size (bp)	Annealing temp. (°C)
T_6	F: CAAGTC	1341-1645	305	53.8
	CCACCGAGGAA	-	-	-
	R: AGACCACCC	-	-	-
	TTGGGTTACAT	-	-	-

The position was calculated according to duck THRSPα gene sequence

Corresponding Author: Guo-Hong Chen, Animal Science and Technology, Yangzhou University, Yangzhou 225009, China

SNPs identification with PCR-SSCP technique and sequencing confirmation: Goose genomic DNA was extracted from blood sample and diluted to 100 ng μL^{-1}. PCR was performed in 20 μL mixture containing 100 ng of goose genomic DNA, 10×PCR buffer (Mg^{2+} free) 2 μL, 25 mmol L^{-1} $MgCl_2$ 1 μL, 2.5 mmol L^{-1} of each dNTP 1 μL, 10 μmol L^{-1} primers 1 μL, 0.2 μL (1.0 U) Taq DNA polymerase (TakaRa Biotechnology Dalian Co., Ltd.), 100 ng μL genomic DNA 1 μL and 12.8 μL ddH_2O.

PCR was run with the following procedure: 95°C for 5 min followed by 35 cycles of 50 sec at 94°C, 50 sec at annealing temperature 53.8°C, 50 sec 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 star and Align IR 2.0 software.

RESULTS AND DISCUSSION

The sequence confirmation of goose THRSPα gene exon 2 fragment: Three individuals of each genotype were selected randomly to clone and sequence. The results were shown in Fig. 1.

The identity of sequences amplified between goose and duck was 89.00%.

The genetic variation of goose THRSPα gene exon 2 fragment: About 6 SNPs were detected by this primer. The mutations were G19A, C62T, G70C, A136G, A198G and G244A, respectively (Fig. 2) (the number was calculated from the first base of amplified fragment by the primer).

Six genotypes AA, BB, CC, AB, AC and BC were generated among 4 populations (Fig. 3), the genotypes distribution and gene frequency of the polymorphic primer in 4 populations were shown in Table 2.

Two exons in goose THRSPα gene were amplified according to duck THRSPα gene sequences. The results suggested that the sequence of THRSPα gene exon 2 was highly conserved between goose and duck. The role of THRSPα gene at fat induced generation have been studied extensively. Compe *et al.* (2001) report that the amount of the Spot 14 protein is closely related to the full expression of enzymes involved in the glycolytic and lipogenic pathways. In mammals, THRSP is a small acidic protein that is predominately expressed in lipogenic tissue (i.e., liver, abdominal fat and the mammary gland).

This gene has been postulated to play a role in lipogenesis, since it responds to thyroid hormone stimulation, high glucose levels and it is localized to a chromosomal region implicated in obesity. Wang *et al.* (2004) report that the THRSPα locus is associated with abdominal fat traits in resource population.

The polymorphic alleles involving a variable number of tandem repeats were discovered in the putative protein

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goose-AA  CAAGTCCCACCGAGGAACGTCCTTCGTGACCAGCACAGAGCTTGGCCGCCTGCCCGCCGGGCGCTGCCTCTACCGTCCAA   80
goose-CC  ------------------a-------------------------------------------------g---------   80
goose-BB  ------------------------------------------------------------t-------g---------   80
duck      ----------------------ct---------g-t-----c-------g-----------------gg---ag----   80

goose-AA  GTTGCCCCGCGGCCGATCCCGCCGGCTTCGTCTCACCTCCGTCCCCTGGAAGTGAAATTTCCCTGCAGCAGGGCCGAAGC  160
goose-CC  ------------------------------------------------------------------------------  160
goose-BB  ----------------------------------------------------------g-------------------  160
duck      -c-----------------------------------------g-----a-g----------------------a-----  160

goose-AA  AAGTGTTCCGTGTTATTGCTTTGTAGAAGGTATCCATGACTTCGAAGCAATGCTTTTTTTTTTTTTT.....TT  229
goose-CC  ------------------------------------a-------------------------.........  224
goose-BB  ------------------------------------a-------------------------.....--  229
duck      ---c----a-------c---c----t----c---tg-a----t-------g------a----a----atgttatgtta--  240

goose-AA  ..TTTTCTGCGTGTATCTAAACCACCTCCC.ACCGGTCTCTGATGATGTAACCCAAGGGTGGTCT.....  291
goose-CC  ..----------g---------------.--------------------------------.....  286
goose-BB  tt----------g---------------.--------------------------------.....  293
duck      tta---t-------g------tt-------c--t--------g----------------------.....  305
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Fig. 1: The sequences of goose THRSPα gene exons fragment and the alignment with duck, means the identical base; goose-AA, goose-BB and goose-CC represent different homozygotes

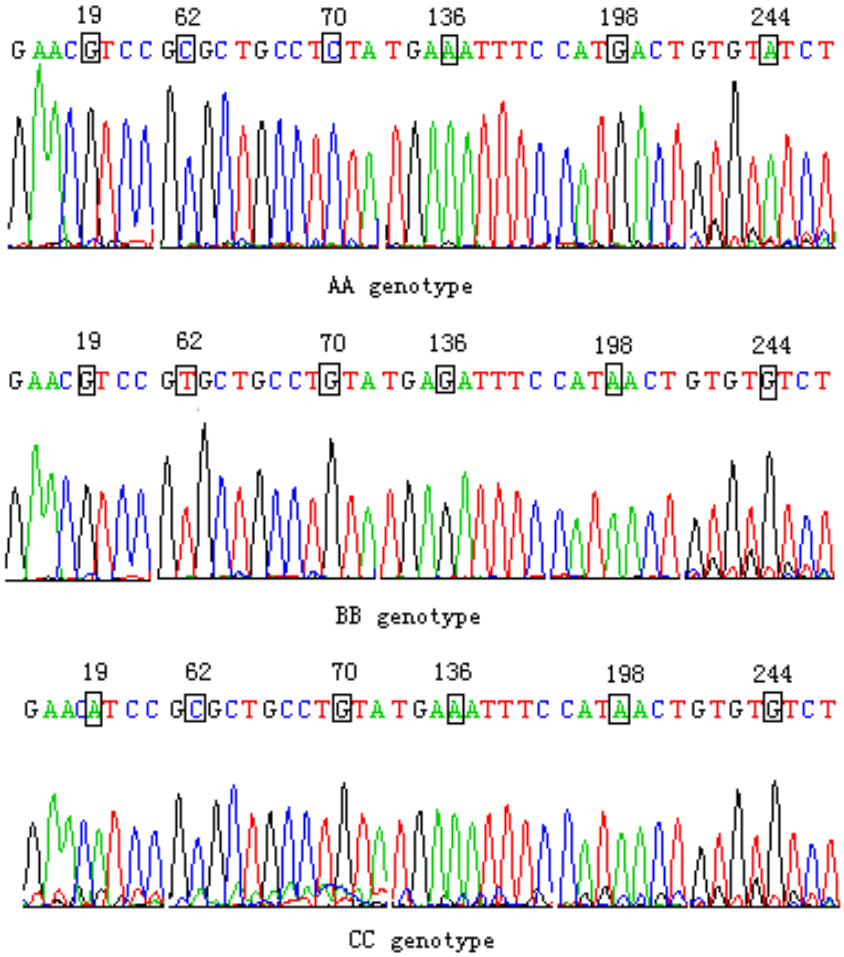

Fig. 2: Chromatograms showing sequence variations at each position within the sequence of THRSPα gene exon 2, bases in frame were the mutation positions

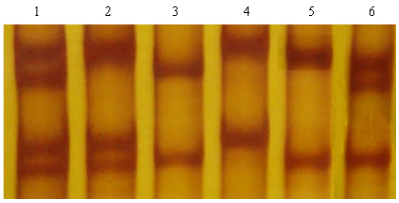

Fig. 3: Different genotypes visualized by electrophoresis. 5, AA genotype; 4, BB genotype; 3, CC genotype; 2, AB genotype; 1, BC genotype 6, AC genotype

coding region of the duplicated chicken THRSPα (9 bp) and THRSPβ (6 or 12 bp) genes. Zhan *et al*. (2007) cloned the full length of THRSPα gene of ducks and was used to detect the polymorphism in THRSPα intron. The association of mutation locus with growth and body compositions of 238 Pekin ducks was investigated by PCR-RFLP method and the C239A in THRSPα intronwas cut by enzyme Sac I. In the study, 6 SNPs were detected with the method of PCR-SSCP and sequencing. Considering the essential effects of THRSPα 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. Zi goose is a small egg-type goose and Wanxi white goose, Zhedong white goose are medium dual type goose.

Shitou goose was the largest meat-type goose. The proportion of AA genotype was dominant in all 4 Chinese indigenous geese and AB genotype was second dominant. Frequency of B allele more than C allele in Zi goose, Wanxi white goose and Zhedong white goose which was significantly different from Shitou goose.

This result suggested that goose THRSPα gene may affect the traits of different populations in different ways. And further studies should be conducted in the relationships of THRSPα gene polymorphism and carcass traits in Goose.

Table 2: Sample size, genotype and gene frequency in 4 goose populations for the polymorphic primer pairs

Population	Sample size	Genotype frequency						Allele frequency			χ^2
		AA	BB	CC	AB	AC	BC	A	B	C	
Zi goose	59	39 (0.661)	1 (0.017)	1 (0.017)	12 (0.203)	6 (0.102)	0 (0.000)	0.813	0.119	0.068	2.12^{ns}
Wanxi white goose	66	29 (0.439)	9 (0.136)	3 (0.046)	20 (0.303)	4 (0.061)	1 (0.015)	0.621	0.296	0.083	6.07^{ns}
Zhedong white goose	71	53 (0.746)	3 (0.042)	2 (0.028)	7 (0.099)	6 (0.085)	0 (0.000)	0.838	0.092	0.070	5.26^{ns}
Shitou goose	67	44 (0.657)	0 (0.000)	4 (0.060)	4 (0.060)	14 (0.208)	1 (0.015)	0.791	0.037	0.172	1.21^{1ns}

χ^2 values means the test values of different genotype to Hardy-Weinberg balance; Means with ns in column are not significantly different ($p>0.05$)

CONCLUSION

This experiment was conducted to study the Polymorphism on exon 2 of THRSPα gene in goose. Six SNPs were found in the sequence of THRSPα gene and 6 genotypes were detected in 4 goose populations. All the populations were in Hardy-Weinberg equilibrium at this polymorphic site ($p>0.05$). The results confirmed that there were polymorphisms in the exon 2 of THRSPα gene.

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