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Polymorphism identification in the goat *THRSP* gene and association analysis with growth traits

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Abstract

In this study, we reported the analysis of *THRSP* gene polymorphisms in 610 goats of three breeds: Xinong Saanen (SN), Guanzhong (GZ) and Boer (BG). We identified new allelic variant: P2-G39294A (GenBank acc. no. JN618075) in the three goat breeds. At P2 locus, GG, GA and AA genotypes were found in the three goat breeds. The frequencies of G allele were 0.54-0.55 and frequencies of A allele were 0.46-0.45, and the PIC was 0.37. The SNP locus was in Hardy-Weinberg disequilibrium in Boer goat breed ($P<0.05$). Association of polymorphisms with growth traits was done at P2 locus in Boer goat breed. The result showed that AA genotype had remarkable growth traits at P2 locus ($P<0.05$). Therefore, these results suggest that *THRSP* gene is a strong candidate gene that affects growth traits in goat.

Keywords: variant, Boer goat, *THRSP*, growth traits

Introduction

The physiological regulation of growth traits is under the control of multiple genes, which may be important candidates for unraveling the genetic variation in economically relevant traits in farm animals (Wu *et al.* 2008, An *et al.* 2010). Availability of genetic information, particularly for those loci which affect performance traits may be important tools in breeding program. The use of molecular genetic technologies potentially offer a way to select a breeding animal for a wide range of traits at an early age (even embryos) and to enhance reliability in predicting the mature phenotype of the individual (Selvaggi *et al.* 2009).

Thyroid hormone responsive (*THRSP*) gene encodes a small acidic protein expressed predominately in the lactating mammary gland, fat, and liver (Zhan *et al.* 2006). *THRSP* mRNA levels are greatly increased by carbohydrate feeding or insulin-injection and decreased by high plasma glucagon levels or by feeding a diet rich in polyunsaturated fatty acids (Jump *et al.* 1993). Invernizzi *et al.* (2010) reported that expression of *THRSP* decreased by day 7 with both diets (saturated lipid and a blend of fish/soybean oil) and returned to basal levels by day 21 in bovine mammary tissue. *THRSP* is an important transcription factor that controls the expression of several lipogenic genes (Towle *et al.* 1997). In humans, *THRSP* gene was shown to be associated with obesity (Chagnon *et al.* 1998), growth, and differentiation of breast cancer cells (Sanchez-Rodriguez *et al.* 2005). The messenger RNA expression of *THRSP* was highly correlated

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with intramuscular fat content of an individual in Wagyu × Hereford cattle (Wang *et al.* 2009). Research showed that the *THRSP* gene has impact on chicken fat metabolism (Breuker *et al.* 2010) and SNPs could be used in molecular marker assistant selection (MAS) as a genetic marker for the chicken and pig growth traits (Wang *et al.* 2004, d'Andre Hirwa *et al.* 2010, Chen *et al.* 2011). However, up to now, the research about SNPs of the *THRSP* gene was not reported in goat. The objectives of the present study were to identify SNPs of *THRSP* gene in three goat breeds and to evaluate associations between the polymorphisms and growth traits in Boer goats.

Material and methods

Sample collection and DNA extraction

Blood samples were obtained from 610 goats belonging to three breeds: Xinong Saanen (SN, n=196), Guanzhong (GZ, n=198) and Boer (BG, n=216). Xinong Saanen and Guanzhong are dairy breeds, while Boer is a very important breed for mutton production in China. They were reared, respectively, in Qianyang county of Shaanxi province (34° 39' N, 107° 7' 48" E and 830 m altitude), Zhouzhi county of Shaanxi province (34° 14' N, 108° 37' E and 1 000 m altitude) and Linyou county of Shaanxi province (34° 42' N, 107° 48' E and 740 m altitude). The growth traits (3-months old) of 216 Boer goats from the same farm were recorded for statistical analysis. The following traits were evaluated: body weight, withers height, body length and chest girth. Five milliliters blood was collected aseptically from the jugular vein in a tube containing anticoagulant ACD (citric acid : sodium citrate : dextrose - 10 : 27 : 38). All samples were delivered back to the laboratory in an ice box. The genomic DNA was extracted from white blood cells using standard phenol-chloroform extraction protocol.

PCR conditions

According to bovine *THRSP* gene (GenBank acc. no. NC_007330), five pairs of primers were designed to amplify goat *THRSP* gene and screened for polymorphisms. Only Primer 2 (P2 locus) has polymorphism. The 25 µL reaction mixture contained 50 ng genomic DNA, 0.5 µM of each primer, 1× Buffer (including 1.5 mm $MgCl_2$), 200 µM dNTPs and 0.625 U Taq DNA polymerase (MBI). The cycling protocol was 5 min at 95 °C, 35 cycles of denaturing at 94 °C for 30 s, annealing at 57 °C for 30 s, extending at 72 °C for 35 s, with a final extension at 72 °C for 10 min.

Table 1
Primer sequences and information of *THRSP* gene

Name	Sequence, bp	Temp., °C	Amplicon	Product size, bp
Primer 1	F: 5- CAA GAA CTG CCT GCT GAC-3 R: 5-ACC ATT ACC TTT CCT ACA CG-3	51	Exon 1	440
Primer 2	F: 5-CCA AAC TGC CAA CTT CAA CC-3 R: 5-TCA CTG CTC TGC CAT CCC TA-3	57	Intron 1 (3162-3587)	426
Primer 3	F: 5-GCA TCT GGT CCC ATC ACT TC-3 R: 5-GGC ACT CAG CCT TCT TCA CA-3	57	Intron 1 (4045-4275)	231
Primer 4	F: 5-TGC CAA CAA AGG TCC GTC TA-3 R: 5-AAT CCC TCC CAG CAT CAG TC-3	58	Intron 1 (4232-4476)	245
Primer 5	F: 5-TCA TCA CTG CGT CAC CGT TAG-3 R: 5-TCG GCT TCT TAG TTC TGT AGG-3	56	Exon 2	360

SNP detection and DNA sequencing

PCR products (6 µL) were mixed with 8 µL denaturing solution (95 % formamide, 25 mm EDTA, 0.025 % xylene cyanole and 0.025 % bromophenol blue), heated for 10 min at 98 °C and chilled on ice. Denatured DNA samples were subjected to PAGE (80 × 73 × 0.75 mm) in 1 × TBE buffer and constant voltage (190 V) for 3.5 h. The gel (29:1 acrylamide:bis) was stained with 0.1 % silver nitrate (He *et al.* 2011). After the polymorphisms had been detected, amplicons representing unique banding patterns were sequenced in both directions in ABI 377 DNA analyzer (Applied Biosystems, Foster City, CA, USA) and the sequences were analyzed with Lasergene 7.1 (DNAStar, Madison, WI, USA) and BLAST (National Center for Biotechnology Information, Bethesda, MD, USA).

Statistical analysis

The allelic frequency, heterozygosity (He), Hardy-Weinberg equilibrium and polymorphism information content (PIC) were calculated using Genepop software 4.0 (Rousset 2008). The software SPSS 16 (SPSS Inc., Chicago, IL, USA) was used to analyze the relationship between genotypes and growth traits in goats. Adjusted linear model:

$$Y_{ijlm} = \mu + S_i + D_{ij} + G_l + E_{ijlm} \quad (1)$$

where Y_{ijlm} is the trait measured on each of the *ijlm*-th animal, μ is the overall population mean, S_i is the fixed effect associated with the *i*-th sire, D_{ij} is the fixed effect associated with *j*-th dam with sire *i*-th, G_l is the fixed effect associated with *l*-th genotype, and E_{ijlm} is the random error. Effects associated with farm and season of birth (spring vs fall) are not matched in the linear model, as the preliminary statistical analyses indicated that these effects did not have a significant influence on variability of traits in the analyzed breeds.

Results and discussion

According to international practice and reference to (Brka *et al.* 2010, Kulig *et al.* 2010, An *et al.* 2011) about the naming of SSCP patterns, In P2 locus, different SSCP patterns were named GG, GA and AA genotypes. The alleles were named G and A, and GG, GA and AA genotypes were found in the three goat breeds. The frequencies of G allele were 0.54-0.55 and frequencies of A allele were 0.46-0.45, and the PIC was 0.37 (Table 2). According to the classification of PIC (low polymorphism if PIC value <0.25, moderate polymorphism if 0.25 <PIC value <0.50, and high polymorphism if PIC >0.50) (Ma *et al.* 2011), the three goat breeds at P2 locus had moderate genetic diversity. The P2 locus were in Hardy-Weinberg equilibrium in SN and GZ breeds (P>0.05), which showed the genotypic frequencies has not been affected by selection, mutation or migration in both goat breeds. In BG breed, the P2 locus were in Hardy-Weinberg disequilibrium (P<0.05), which suggested the genotypic frequencies were subjected to selection, mutation or migration in the breed. Only Boer goat was not in Hardy-Weinberg equilibrium at P2 locus which may be caused by specific selection for mutton production which affects the genotypes directly or indirectly. Hence, the goat *THRSP* gene was considered to have positive effects on growth traits. At P2 locus, the different electrophoresis patterns of PCR products were sequenced in both directions, and comparisons among these nucleotide

sequences of difference genotypes indicated that one base substitution (P2-G39294A, GenBank acc. no. JN618075) were detected. In order to study the possible association between the carriers of different genotypes and the trait values, growth traits from 3-months old were analyzed in Boer goat breed (Table 3). At P2 locus, the does with AA genotype had greater body weight than those with GG and GA genotypes ($P<0.05$); in addition, the does with AA genotype had greater chest girth than those with GA genotype ($P<0.05$). We consider that these associations can be explained by the following two possible reasons: (1) Although this mutation of P2 locus does not concern the coding region, it possibly influences the stability of the mRNA, can affect the mechanism of mRNA deadenylation and degradation (Gallie & Young 1994, Clement *et al.* 2001). (2) Linkage disequilibrium with the causal mutation possibly affects the variation of the growth traits in goat. If this polymorphism is in linkage disequilibrium with a gene affecting the variation of the growth traits, segregation based on marker alleles would result in phenotypic differences (Van der Werf *et al.* 2007). The mutations found might not be the causal mutation by themselves, but might be in linkage disequilibrium with the causal mutation which could affect either the *THRSP* gene or other genes near to the *THRSP* locus. Chen *et al.* (2011) implied the association of polymorphism at G123A and A308G sites in *THRSP* gene with lipogenesis capability of pigs and their important regulating role in the expression of lipogenesis genes in pigs. Expression analysis by real-time quantitative PCR showed that *THRSP* paralogs in ducks were more actively transcribed in fat tissues than in liver (Zhan *et al.* 2006). Wang *et al.* (2004) reported *THRSP*α locus was associated with abdominal fat traits in a broiler × Leghorn resource population. The A213C and 9 bp insertion-deletion of exon 1 in the *THRSP* gene was found to be associated with body weight in the chicken (Cao *et al.* 2007).

Table 2
Genotypic distribution, allelic frequencies of P2 locus in three goat breeds

		Breed		
		SN	GZ	BG
Genotype	GG	59	62	56
	GA	97	90	125
	AA	40	46	35
Allele	G	0.55	0.54	0.55
	A	0.45	0.46	0.45
He		0.49	0.45	0.59
PIC		0.37	0.37	0.37
Equilibrium χ^2 test		$P>0.05$	$P>0.05$	$P<0.05$

Table 3
Effects of *THRSP* genotypes on growth traits at P2 locus in Boer goats

Genotype	Body weight, kg	Withers height, cm	Body length, cm	Chest girth, cm
GG	15.00±0.30[a]	44.98±0.43	45.68±0.39	54.63±0.52
GA	14.95±0.20[a]	45.06±0.29	46.07±0.26	54.49±0.35[a]
AA	15.97±0.37[b]	44.63±0.55	45.89±0.50	56.00±0.65[b]

The data are expressed as least square means±standard errors. Values with different superscripts within the same column differ significantly at $P<0.05$.

This supports the notion that further investigation of *THRSP* variation in different goat breeds is needed. Till now, the association of *THRSP* genetic variations with growth traits has not been reported in domestic ruminant. The biochemical and physiological functions, together with the results obtained in our study, indicate that the *THRSP* gene might play important roles affecting growth traits in goat.

In conclusion the mutation (G39294A) we have identified in the intron 1 of *THRSP* gene could be potential genetic markers for growth traits in goats.

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