Mol Biol Rep (2011) 38:3129–3135
DOI 10.1007/s11033-010-9983-7

Molecular characterization and polymorphisms of the caprine Somatostatin (*SST*) and SST Receptor 1 (*SSTR1*) genes that are linked with growth traits

Q. J. Jin · J. J. Sun · X. T. Fang · C. L. Zhang · L. Yang · D. X. Chen · X. Y. Shi · Y. Du · X. Y. Lan · H. Chen

Received: 17 October 2009 / Accepted: 20 January 2010 / Published online: 7 February 2010

Abstract Somatostatin (SST) and its receptors (SSTR1-5) appear to be important in central regulation of many metabolic systems that affect growth, adiposity and nutrient absorption. In this study, we investigated polymorphisms within the caprine *SST* and *SSTR1* genes and determined their relationship with growth traits. As there were no sequence information of the caprine *SST* and *SSTR1* genes, we explored their DNA sequence and genomic organizations. The caprine *SST* gene is organized in two exons and is transcribed into an mRNA containing 351 bp of sequence coding for a protein of 116 amino acids. Its protein sequences showed substantial similarity (97–99%) to its respective orthologs from cattle, human and mouse. We also cloned and sequenced a 1.2 kb DNA fragment which contained the major part of the coding region and 3′ UTR of the caprine *SSTR1* gene. We then detected the polymorphisms in these determined sequences by PCR-SSCP and DNA sequencing methods in 459 goats from four breeds. Four SNPs (GU014693:g.647T>C, GU014693:g.844A>C, GU014693:g.970T>C, GU014693:g.1039T>A), segregating as two haplotypes (T-A-T-T and C-C-C-A), were identified in intron 1 of the caprine *SST* gene and showed the associations to body length and body height ($P < 0.05$). Two SNPs (GU014695:g.801 C>T, GU014695:g.948 C>T) were identified in the caprine *SSTR1* gene. Significant associations between the three genotypes of GU014695:801 C>T and body length, body height, and chest circumference was observed ($P < 0.05$). These results suggest that the caprine *SST* and *SSTR1* genes are strong candidate genes that influence growth traits in goat.

Keywords Goat · *SST* gene · *SSTR1* gene · SSCP · SNP · Association analysis

Introduction

Somatostatin (SST) was original discovered by Brazeau et al. [1] as a hypothalamic factor that inhibits the secretion of growth hormone. SST and its receptors (SSTR) were subsequently found in several extra-hypothalamic areas of the central nervous system [2, 3], pancreas, intestinal tract, stomach, kidney, liver, pancreas, lung and placenta [4–7]. The widespread distribution of SST and its receptors suggest that they exert multiple biological functions. Besides the inhibitory effects on GH-release, SST inhibits the release of a variety of other peptides including prolactin and thyrotropin in the anterior pituitary, gastrin, ghrelin and secretin in gastrointestinal tract, glucagons, insulin and SST itself in pancreas [8–10]. SST has been shown to regulate the rate of nutrient absorption from the gastrointestinal tract by inhibiting the secretion of gastrointestinal hormones, decreasing the secreted volume of digestive enzymes [11, 12]. It also reduces gastrointestinal motility,

Electronic supplementary material The online version of this article (doi:10.1007/s11033-010-9983-7) contains supplementary material, which is available to authorized users.

Q. J. Jin · J. J. Sun · X. T. Fang · C. L. Zhang · L. Yang · D. X. Chen · X. Y. Shi · Y. Du · H. Chen (✉)
Institute of Cellular and Molecular Biology, Xuzhou Normal University, No. 101 Shanghai Road, 221116 Xuzhou, Jiangsu, People's Republic of China
e-mail: chenhong1212@263.net

X. Y. Lan
College of Animal Science and Technology, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwest A&F University, No. 22 Xinong Road, 712100 Yangling, Shaanxi, China

gallbladder contraction and blood flow rates [13]. By controlling digestion and absorption rates, somatostatin can also influence feed conversion, growth, and adiposity traits.

The diverse effects of somatostatin are mediated by specific, high-affinity membrane bound somatostatin receptors (SSTRs) on target tissues. So far, five subtypes of SSTRs, SSTR1, -2, -3, -4, and -5, have been identified which are members of G-protein coupled receptor family [14]. The five Somatostatin receptors (SSTR1-5) are variably expressed throughout numerous tissues ranging from the central nervous system to the endocrine and immune systems [15]. Somatostatin receptor subtype 1 (SSTR1) appears to be important in central regulation of insulin and GH secretion. Kreienkamp et al. [16] reported that the expression of the *SSTR1* gene is essential in the regulation of basal levels of GH in mice. Mice lacking a functional *SSTR1* gene are unable to modulate these basal GH levels in primary pituitary cell cultures and have significantly reduced body weight with growth retardation [17].

Genes that regulate metabolism and energy partitioning have the potential to influence economically important traits in farm animals, as do polymorphisms within these genes. Considering the effects of SST and its receptors on the growth hormone axis and the control of growth, adiposity and nutrient absorption, this study was conducted to explore the molecular characterization and polymorphisms of the caprine *SST* and *SSTR1* genes, and then analyze the association between each genotype and its growth traits among four goat breeds. This will be helpful for conserving, utilizing, and exploiting the genetic resources of goat.

Materials and methods

Animals and data source

Genomic DNA samples were obtained from 459 unrelated goats belonging to four breeds: Boer goat (BE, $n = 111$), Chinese Xuhuai white goat (XH, $n = 108$) and Chinese Haimen goat (HM, $n = 136$), crossing population (BE × XH $n = 104$). They were reared in Jiangsu province of China. DNA samples were extracted from leucocytes according to Müllenbach et al. [18]. The four growth traits (body height, body length, cannon circumference, chest circumference) were measured at 18 months of goats. Body length index (%) (body length/body height × 100), chest circumference index (%) (chest circumference/body height × 100), cannon circumference index (%) (cannon circumference/body height × 100), trunk index (%) (chest circumference/body length × 100) were calculated. Data including eight growth traits for four breeds were summarized by descriptive statistics and were presented in Table 1. Boer goats produced the highest mean values for body height, body length, cannon circumference, chest circumference. However, Haimen goats produced the lowest mean values for the four growth traits (Table 1).

Table 1 Descriptive statistics of the recorded growth traits for four goat breeds

Breeds (N) traits	Mean	SE	Min	Max
BE (111)				
BH (cm)	65.55	0.69	45.00	98.00
BL (cm)	77.00	0.75	52.00	99.00
CaC (cm)	10.30	0.12	6.50	14.00
ChC (cm)	82.61	1.03	50.00	99.00
BLI (%)	118.57	1.52	84.69	177.55
ChCI (%)	126.96	1.78	80.95	182.22
CaCI (%)	15.83	0.21	10.87	22.92
TI (%)	108.43	1.70	57.95	167.31
XH (108)				
BH (cm)	61.36	0.51	49.00	73.00
BL (cm)	70.63	0.74	53.00	84.00
CaC (cm)	8.22	0.06	7.00	9.50
ChC (cm)	77.09	0.80	59.00	93.00
BLI (%)	115.06	0.71	133.33	115.06
ChCI (%)	125.79	1.01	101.61	150.88
CaCI (%)	13.47	0.12	11.29	16.67
TI (%)	109.48	0.83	92.65	145.28
HM (136)				
BH (cm)	56.80	0.31	50.00	67.00
BL (cm)	65.43	0.49	54.00	84.00
CaC (cm)	8.47	0.08	6.00	11.00
ChC (cm)	75.35	0.39	65.00	85.00
BLI (%)	115.21	0.62	93.44	130.77
ChCI (%)	132.94	0.75	115.25	152.94
CaCI (%)	14.91	0.12	10.91	17.24
TI (%)	115.64	0.68	96.34	140.00
BE × XH (104)				
BH (cm)	65.12	0.41	54.00	74.00
BL (cm)	73.73	0.69	60.00	88.00
CaC (cm)	8.63	0.08	7.50	11.00
ChC (cm)	79.23	0.79	62.00	100.00
BLI (%)	113.23	0.80	94.37	133.33
ChCI (%)	121.67	0.94	94.29	143.75
CaCI (%)	13.26	0.10	11.43	16.92
TI (%)	107.62	0.69	92.86	125.00

XH Xuhuai goat, *BE* Boer goat, *HM* Haimen goat, *BE* × *XH* Boer goat × Xuhuai goat, *BH* body height, *BL* body length, *CaC* cannon circumference, *ChC* chest circumference, *BLI* body length index, *ChCI* chest circumference index, *CaCI* cannon circumference index, *TI* trunk index, *N* number of observations, *Mean* means of traits, *SE* standard error, *Min and Max* minimum and maximum values

PCR amplification

Comparative alignments of amino-acid sequences and nucleotide sequences from cattle and sheep were used to design PCR primers (PS0 and PR0) that would amplify the entire coding region and intron of caprine *SST* gene and a 1.2 kb segment which contained the major part of the coding region of the caprine *SSTR1* gene (Supplementary Table S1). The PCR products were subcloned to T-vector (Promega) and sequenced in both directions in ABI PRISM 377 DNA sequencer (Applied Biosystems). Eight primer pairs was subsequently designed according to the sequences of caprine *SST* and *SSTR1* genes we determined, to detect polymorphisms in these sequences (Supplementary Table S1).

For all assays processed in this study, the same conditions were used. 50 ng genomic DNA, 0.5 μM of each primer, 1× buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs and 0.625 units of Taq DNA polymerase (MBI). The cycling protocol was 5 min at 94°C, 35 cycles of 94°C for 30 s, annealing for 35 s, 72°C for 35 s, with a final extension at 72°C for 10 min.

SSCP and DNA sequencing

SSCP method was used to scan mutations within the amplified regions. Aliquots of 5 μl PCR products were mixed with 5 μl denaturing solution (95% formamide deionized, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled in ice immediately. Denatured DNA was subjected to 10% PAGE (polyacrylamide gel electrophoresis) in 1× TBE buffer and constant voltage (150 V) for 15 h at a constant temperature of 4°C. The gel was stained with silver nitrate and visualized with 2% NaOH solution (containing 0.1% formaldehyde) [19]. The PCR products which represented different PCR-SSCP genotypes, including both homozygous and heterozygous genotypes were purified with the GenElute PCR DNA Purification Kit (Sigma–Aldrich Corporation, USA) and sequenced using the ABI 377 sequencer from both directions (Applied Biosystems, USA). Sequences were aligned using web based CLUSTAL-W (http://www.ebi.ac.uk/clustalw/index.html) program.

Association studies and statistical analysis

Gene frequencies were determined for each breed by direct counting. A χ^2-test was applied to assess statistical significance using the software of SPSS V17.0 (SPSS Inc., USA). The effects associated with season of birth (spring vs. fall), age of dam and sire were not included into the linear model, as the preliminary statistical analyses indicated that these effects did not significantly influence on variability of traits in the population. Therefore, the effects of genotype on the traits were analyzed by the least-squares method as applied in the general linear model (GLM) procedure of SPSS according to the following statistical model:$Y_{ij} = \mu + B_i + G_j + E_{ij}$, where Y_{ij} was the trait measured on each of the ijth animal, μ was the overall mean, B_i was the type of the ith breed, G_j was the type of the jth genotype and E_{ij} was the random error.

Results

Molecular characterization and polymorphisms of the caprine *SST* gene

We cloned and sequenced a 1,408 bp fragment including the entire coding region and intron of the caprine *SST* gene. The sequence was deposited in GenBank with accession number GU014693. As summarized in Supplementary Fig. S1, the caprine *SST* gene is divided into two exons. Exon 1 contains the translation start site that is predicted by the programs HMMGene and Genscan (http://genius.embnet.dkfz-heidelberg.de). Thus, the caprine *SST* gene is transcribed into an mRNA containing 351 bp of sequence coding for a protein of 116 amino acids. The exon–intron boundaries of the caprine *SST* gene were predicted by aligning the goat and cattle DNA sequences with the known structure of the bovine *SST* gene (Supplementary Table S2). We found that exons of the caprine *SST* gene followed the AG-GT rule for splice acceptor and donor sites. The exon–intron organization of the gene is perfectly conserved between the caprine and the bovine *SST* gene. Intron sizes were precisely determined and containing 842 bp. Alignment of the caprine SST cDNA with corresponding coding sequences from other mammalian species showed similarity of 98% for cattle, 90% for humans and 88% for mice. On the protein level, the caprine amino acid sequence revealed 99, 99, and 97% identity with cattle, human and mouse, respectively, which is demonstrated in Supplementary Fig. S2.

Three primer pairs were subsequently designed according to the sequence of the caprine *SST* gene sequenced here for PCR-SSCP and DNA sequencing focused on identifying SNPs within the exon 1, exon 2 and intron 1. In total 459 unrelated goats belonging to four goat breeds: Boer goat (BE, $n = 111$), Chinese Xuhuai white goat (XH, $n = 108$) and Chinese Haimen goat (HM, $n = 136$), crossing population (BE × XH, $n = 104$) were chosen for scan mutations by PCR-SSCP and DNA sequencing methods. Only in the PS2 locus, three SSCP genotypes were identified and denominated as PS2-A, PS2-B and PS2-AB genotypes. The sequence analysis of the three

genotypes revealed four SNPs: GU014693:g.647T>C, GU014693:g.844A>C, GU014693:g.970T>C and GU014693:g.1039T>A. They formed two consistent haplotypes (T-A-T-T and C-C-C-A). Three genotypes identified in the SSCP analyses for the PS2 locus showed homozygous PS2-A, PS2-B and heterozygous PS2-AB by haplotypes T-A-T-T and C-C-C-A, respectively.

Based on SSCP and responsible sequence variations, the genotype and haplotypes frequencies were analysed (Table 2; Fig. 1). The average frequency of the haplotype T-A-T-T in all populations ($n = 459$) is 0.7031. To test the hypothesis that the polymorphisms within the caprine *SST* gene would contribute to the variation of growth traits, we performed an association analysis between the four SNPs and body height, body length, cannon circumference, chest circumference, body length index (%), chest circumference index (%), cannon circumference index (%), and trunk index (%). In 459 goats, the association analysis of the PS2 locus indicated a significant effect of PS2 locus on body length, body height, cannon circumference, chest circumference and cannon circumference index. Genotype PS2-A was associated with a decreased body length, body height, cannon circumference and chest circumference compared with genotypes P2-B and P2-AB (Table 3). Moreover, haplotype C-C-C-A of the PS2 locus had a higher frequency in Boer goat which produced the highest mean values for body height, body length, cannon circumference, chest circumference compared with the other goat breeds.

Molecular characterization and polymorphisms of the caprine *SSTR1* gene

We cloned and sequenced a 1,194 bp fragment contained the major part of the coding region of the caprine *SSTR1* gene. The sequence was deposited in GenBank with accession number GU014695. As summarized in Supplementary Fig. S3, the determined sequence of the caprine *SSTR1* gene contained the major part of the coding region and 3′-UTR that is predicted by the programs HMMGene

Table 2 Genotype distribution and haplotypes frequency of PS2 locus

Breed	Observed genotype			Total	Haplotypes frequency	
	PS2-A (TT-AA-TT-TT)	PS2-AB (TC-AC-TC-TA)	PS2-B (CC-CC-CC-AA)		T-A-T-T	C-C-C-A
BE	41	49	21	111	0.5901	0.4099
XH	51	49	8	108	0.6991	0.3009
HM	109	25	2	136	0.8934	0.1066
BE × XH	40	51	13	104	0.6298	0.3702

XH Xuhuai goat, *BE* Boer goat, *HM* Haimen goat, *BE* × *XH* Boer goat × Xuhuai goat

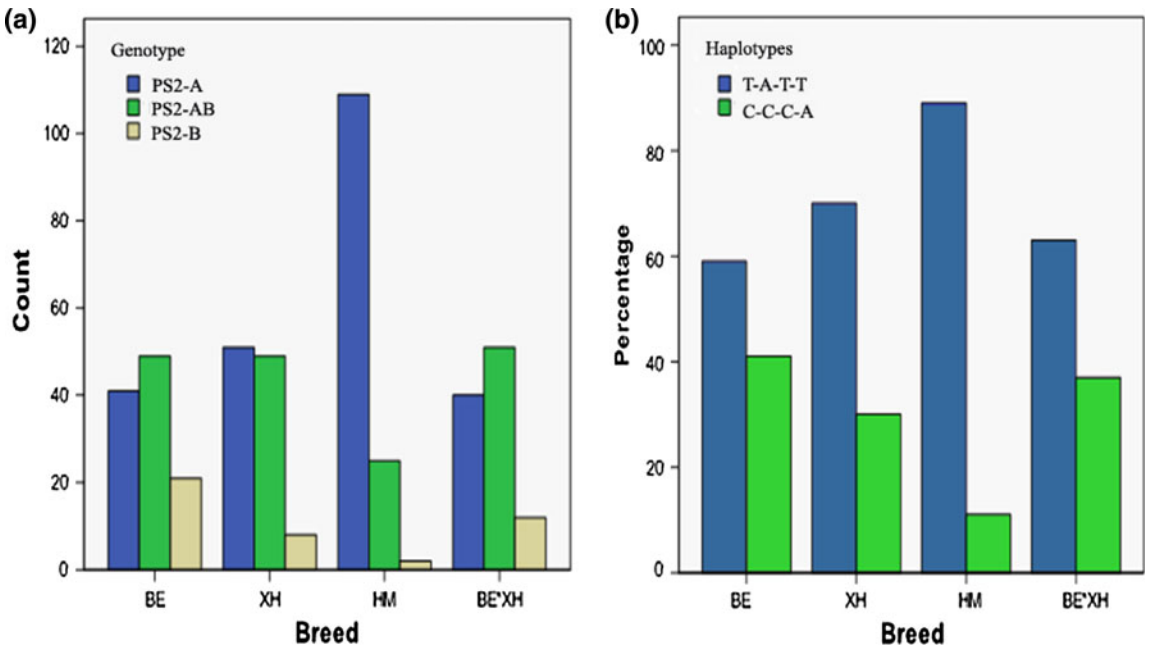

Fig. 1 Genotypic (**a**) and haplotypes (**b**) distribution of PS2 locus within the caprine *SST* gene in four goat breeds. *XH* Xuhuai goat, *BE* Boer goat, *HM* Haimen goat, *BE* × *XH* Boer goat × Xuhuai goat, Count means the genotype number

Table 3 Association analysis of the PS2 locus with growth traits in goats (Boer goat, Xuhuai white goat and Boer goat × Xuhuai goat)

Growth trait	Genotype (means ± standard error of means)			*P* value
	PS2-A (241)	PS2-AB (174)	PS2-B (44)	
BH (cm)	60.884 ± 0./400[a]	62.897 ± 0.444[b]	63.250 ± 1.203[b]	0.002
BL (cm)	70.185 ± 0.556[a]	72.644 ± 0.603[b]	72.409 ± 1.094[ab]	0.008
CaC (cm)	8.795 ± 0.077[a]	8.882 ± 0.092[a]	9.421 ± 0.227[b]	0.030
ChC (cm)	77.452 ± 0.500[a]	79.704 ± 0.682[b]	78.364 ± 1.542[a]	0.009
BLI (%)	115.388 ± 0.626	115.737 ± 0.778	115.580 ± 2.131	0.945
ChCI (%)	127.779 ± 0.767	127.085 ± 0.974	125.089 ± 2.850	0.445
CaCI (%)	14.489 ± 0.110	14.159 ± 0.131	15.068 ± 0.435	0.011
TI (%)	111.152 ± 0.675	110.340 ± 0.920	108.902 ± 2.271	0.457

BH body height, *BL* body length, *CaC* cannon circumference, *ChC* chest circumference, *BLI* body length index, *ChCI* chest circumference index, *CaCI* cannon circumference index, *TI* trunk index

Table 4 Genotype and allele frequencies of the SNPs detected in the caprine *SSTR1* gene

Breeds	GU014695:801C>T					GU014695:948C>T			
	Genotype frequencies			Allele frequencies		Genotype frequencies		Allele frequencies	
	CC	TT	CT	C	T	CC	CT	C	T
BE (111)	7	56	48	0.248	0.752	69	42	0.811	0.189
XH (108)	27	45	36	0.398	0.602	74	34	0.843	0.157
HM (136)	43	34	59	0.375	0.625	57	79	0.710	0.290
BE × XH (104)	7	45	52	0.284	0.716	78	26	0.875	0.125

XH Xuhuai goat, *BE* Boer goat, *HM* Haimen goat, *BE* × *XH* Boer goat × Xuhuai goat

and Genscan (http://genius.embnet.dkfz-heidelberg.de). The partial predicted protein consists of 281 amino. Alignment of caprine *SSTR1* coding sequence with corresponding coding sequences from other mammalian species showed similarity of 99% for sheep, 98% for cattle and 92% for human. On the protein level, caprine amino acid sequence revealed 98, 98, and 97% identity with human, cattle, and sheep, respectively.

Five primer pairs (PR1-PR5) were subsequently designed according to the sequence of caprine *SSTR1* gene we determined, to detect polymorphisms in this sequence. In PR4 locus, three SSCP genotypes were identified and denominated as PR4-CC, PR4-TT and PR4-CT genotypes. Sequence analysis showed that the three SSCP genotypes were caused by C to T mutation at position 801(according to GU014695) (Supplementary Fig. S3). Two SSCP genotypes were identified in PR5 locus, designated as PR5-CC, PR5-CT. The sequence analysis of two genotypes revealed one SNP: 948 C>T (according to GU014695) (Supplementary Fig. S3). SNP GU014695:801C>T was a synonymous mutation and SNP GU014695:948 C>T was in 3′-untranslated regions of the caprine *SSTR1* gene. No polymorphism was detected in other loci. The genotypic and allelic frequencies of the four breeds are given in Table 4.

The association of SNPs with the goat growth traits was analyzed (Table 5). The results indicated that there existed a relationship between genotypes of PR4 locus and growth trait: Genotype PR4-CC was associated with a decreased body length, body height and chest circumference compared with genotypes PR4-TT and PR4-CT ($P < 0.05$). The genotypes of PR5 locus did not show any significant association with goat growth traits ($P > 0.05$).

Discussions

Body height, body length, chest circumference and cannon circumference are four main growth traits which have important impacts on the production of goat meat and pelt. Therefore, breeding for optimal growth traits and larger gains is a major consideration in goat breeding programs. Most genetic variation is represented by single nucleotide polymorphisms and many of them are believed to cause phenotypic differences between individuals. Identification of causative mutations that affect growth traits will greatly enhance the progress towards this goal.

Four breeds of goats (Boer goat (BE, $n = 111$), Chinese Xuhuai white goat (XH, $n = 108$) and Chinese Haimen goat (HM, $n = 136$), crossing population (BE × XH

Table 5 Association analysis of PR4 and PR5 loci in *SSTR1* gene with growth traits in goats (Boer goat, Xuhuai white goat and Boer goat × Xuhuai goat)

Growth trait	Locus PR4 (Lsmean ± SE)				Locus PR5 (Lsmean ± SE)		
	PR4-CC (84)	PR4-TT (180)	PR4-CT (195)	*P* value	PR5-CC (278)	PR5-CT (181)	*P* value
BH (cm)	59.893 ± 0.617[a]	62.539 ± 0.475[b]	62.113 ± 0.463[b]	0.005	62.169 ± 0.352	61.420 ± 0.520	0.217
BL (cm)	68.643 ± 0.964[a]	72.375 ± 0.624[b]	71.523 ± 0.388[b]	0.003	71.685 ± 0.499	70.785 ± 0.617	0.257
CaC (cm)	8.583 ± 0.128	9.061 ± 0.096	8.859 ± 0.087	0.073	8.920 ± 0.073	8.840 ± 0.097	0.268
ChC (cm)	77.095 ± 0.779[a]	79.461 ± 0.686[b]	77.967 ± 0.602[b]	0.013	78.750 ± 0.517	77.845 ± 0.626	0.506
BLI (%)	114.497 ± 0.929	116.081 ± 0.861	115.487 ± 0.716	0.513	115.535 ± 0.660	115.545 ± 0.700	0.992
ChCI (%)	129.155 ± 1.142	127.482 ± 0.955	126.234 ± 1.018	0.221	127.079 ± 0.754	127.533 ± 1.027	0.717
CaCI (%)	14.353 ± 0.168	14.555 ± 0.150	14.323 ± 0.134	0.456	14.402 ± 0.114	14.446 ± 0.137	0.805
TI (%)	113.269 ± 1.234	110.419 ± 0.853	109.685 ± 0.845	0.058	110.556 ± 0.692	110.739 ± 0.874	0.869

BH body height, *BL* body length, *CaC* cannon circumference, *ChC* chest circumference, *BLI* body length index, *ChCI* chest circumference index, *CaCI* cannon circumference index, *TI* trunk index, *Lsmean* ± *SE* means ± standard error of means

$n = 104$)), were applied to screen potential SNPs related to growth trait in the caprine *SST* and *SSTR1* genes based on SSCP and sequencing methods. Two common haplotypes, T-A-T-T and C-C-C-A which consisted with four SNPs (GU014693:g.647T>C, GU014693:g.844A>C, GU014693:g.970T>C and GU014693:g.1039T>A) were found in intron 1 of the caprine *SST* gene. No SNP was detected in the coding region of the caprine *SST* gene is in agreement with our results from comparison of caprine, human, mouse, and bovine cDNA and amino acid sequences demonstrating that the *SST* gene is highly conserved across species.

In our association study, we found indication supporting a trait relationship of the sequence variation found in PS2 locus of the caprine *SST* gene with body length, body height, cannon circumference and chest circumference. Individuals with genotype PS2-B and PS2-AB showed significantly increased body length, body height, cannon circumference and chest circumference compared with individuals with genotype PS2-A in 459 goats. However, the SNPs studied here are all in introns 1 of the caprine *SST* gene, so they may not be causal mutations. Thus, one may suggest that it could be in linkage disequilibrium with another SNP in the *SST* gene with greater effects on the traits. Moreover, introns have been shown to affect transcriptional efficiency of numerous genes in a variety of organisms [20, 21]. There is a growing number of examples for intronic and 3′ untranslated sequence that appear to play a significant role in regulating the expression level of a gene or in defining its tissue-specific expression pattern. Van Laere et al. [22] found a nucleotide substitution in intron 3 of the insulin-like growth factor-2 gene affecting muscle growth, fat deposition, and heart size in pigs. Sobrier et al. [23] also found a splice defect (c.357 + 2T>C) in introns 3 of human *Hesx1* gene lead to the synthesis of truncated proteins partly or entirely lacking the homeodomain, with no transcriptional repression, as shown by their inability to inhibit Prop1 activity. Thus, it seems conceivable that variations in intron 1 of the gene with potential effects on *SST* gene might affect the goat growth traits.

We were led to pursue the *SSTR1* gene because the important role of SSTR1 in the central regulation of insulin and GH secretion. *SSTR1* gene represents an excellent candidate gene that may contribute to the genetic breeding. We have amplified a 1,195 bp fragment contained the major part of the coding region of the caprine *SSTR1* gene, determined its DNA sequence and screened for its polymorphisms. In a sample consisting of 459 goats, we identified two DNA sequence polymorphisms within the 1,195 bp fragment of the caprine *SSTR1* gene based on SSCP and sequencing methods. We found indication supporting a traits relationship of GU014695:801C>T in PR4 locus with body length, body height and chest circumference of goat. Genotype PR4-CC was associated with a decreased body length, body height and chest circumference compared with genotypes PR4-TT and PR4-CT ($P < 0.05$). GU014695:801C>T was a synonymous mutation and has no effect on the amino acid sequence. It has long been assumed that synonymous SNPs are inconsequential, as the primary sequence of the protein is retained. However, there is now considerable evidence that such mutations can, for example, disrupt splicing and interfere with miRNA binding. Two recent publications suggest involvement of additional mechanisms: modification of protein abundance most probably mediated by alteration in mRNA stability [24] and modification of protein structure and activity [25], probably mediated by induction of translational pausing. Moreover, linkage disequilibrium with a functional polymorphism is also likely to be involved in the underlying mechanism. In PR5 locus, we found this fragment only having two genotypes by detection of SSCP. Genotype TT was not found in our study. It is an interesting phenomenon worth of further investigation. The genotypes of GU014695:948 C>T in PR5 locus

did not show any significant association with goat growth traits ($P > 0.05$). So, we did not suggest that GU014695:948 C>T had a positive effect on caprine growth traits. Although the PR5 locus within the caprine *SSTR1* gene was not considered a DNA marker for caprine growth traits, the novel single nucleotide polymorphism identified by PCR-SSCP, DNA sequencing methods has extended the spectrum of genetic variation in the caprine SSTR1 gene, which may contribute to a better understanding of genetic variation in animal resources.

In this study, we first reported the molecular characterization and polymorphisms in the caprine *SST* and *SSTR1* genes, and their correlation with the growth traits in goat. Significant associations between the genotypes of PS2 locus and PR4 locus and goat growth traits were observed ($P < 0.05$). Although the causative mutation was likely not found in our study, we conclude that either the gene itself affects goat growth traits or it is in linkage disequilibrium with other genes that do. Further investigation of the *SST* and *SSTR1* genes including upstream and down stream control regions, is needed to elucidate molecular mechanisms causing the QTL effects.

Acknowledgments This work was supported by the National "863" Program of the P.R. China (No. 2008AA10Z138), Natural Science Foundation of Jiangsu Province (No. BK2008120), the "13115" Sci-Tech Innovation Program of Shaanxi Province (2008ZDKG-11), Research Fund for the Doctor Program of Higher Education of China (No. 20080712001), Natural science fund for colleges and universities in Jiangsu Province (09KJD180002), Natural Science Foundation of Xuzhou Normal University (07XLA08; KY2007019), and the Young Topnotch Researcher Support Project of Northwest A&F University (No.QNGG-2009-007).

References

1. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science (New York, NY) 179:77
2. Finley JC, Maderdrut JL, Roger LJ, Petrusz P (1981) The immunocytochemical localization of somatostatin-containing neurons in the rat central nervous system. Neuroscience 6:2173
3. Møller LN, Stidsen CE, Hartmann B, Holst JJ (2003) Somatostatin receptors. Bba-biomembranes 1616(1):1–84
4. Reichlin S (1983) Somatostatin (second of two parts). New Engl J Med 309:1556–1563
5. Patel YC, Reichlin S (1978) Somatostatin in hypothalamus, extrahypothalamic brain, and peripheral tissues of the rat. Endocrinology 102:523–530
6. Hokfelt T, Johansson O, Efendic S, Luft R, Arimura A (1975) Are there somatostatin-containing nerves in the rat gut? Immunohistochemical evidence for a new type of peripheral nerves. Cell Mol Life Sci (CMLS) 31:852–854
7. Patel YC (1999) Somatostatin and its receptor family. Front Neuroendocrin 20:157–198
8. Luque RM, Gahete MD, Hochgeschwender U, Kineman RD (2006) Evidence that endogenous SST inhibits ACTH and ghrelin expression by independent pathways. Am J Physiol Endocrinol Metab 291:E395
9. Colturi TJ, Unger RH, Feldman M (1984) Role of circulating somatostatin in regulation of gastric acid secretion, gastrin release, and islet cell function. Studies in healthy subjects and duodenal ulcer patients. J Clin Invest 74:417
10. Lloyd KC, Amirmoazzami S, Friedik F, Chew P, Walsh JH (1997) Somatostatin inhibits gastrin release and acid secretion by activating sst2 in dogs. Am J Physiol 272:1481–1488
11. Schusdziarra V (1988) Physiological significance of gastrointestinal somatostatin. Horm Res 29:75–78
12. Tulassay Z (1998) Somatostatin and the gastrointestinal tract. Scand J Gastroenterol 33:115–121
13. Strowski MZ, Parmar RM, Blake AD, Schaeffer JM (2000) Somatostatin inhibits insulin and glucagon secretion via two receptors subtypes: an in vitro study of pancreatic islets from somatostatin receptor 2 knockout mice. Endocrinology 141:111
14. Akopian A, Johnson J, Gabriel R, Brecha N, Witkovsky P (2000) Somatostatin modulates voltage-gated K+ and Ca2+ currents in rod and cone photoreceptors of the salamander retina. J Neurosci 20:929
15. Weckbecker G, Lewis I, Albert R, Schmid HA, Hoyer D, Bruns C (2003) Opportunities in somatostatin research: biological, chemical and therapeutic aspects. Nat Rev Drug Discov 2:999–1017
16. Kreienkamp HJ, Akgn E, Baumeister H, Meyerhof W, Richter D (1999) Somatostatin receptor subtype 1 modulates basal inhibition of growth hormone release in somatotrophs. Febs Lett 462:464–466
17. Wang XP, Norman M, Yang J, Magnusson J, Kreienkamp HJ, Richter D, DeMayo FJ, Brunicardi FC (2006) Alterations in glucose homeostasis in SSTR1 gene-ablated mice. Mol Cell Endocrinol 247(1–2):82–90
18. Mullenbach R, Lagoda PJ, Welter C (1989) An efficient salt-chloroform extraction of DNA from blood and tissues. Trends Genet 5:391
19. Zhang CL, Wang Y, Chen H, Lan XY, Lei CZ (2007) Enhance the efficiency of single-strand conformation polymorphism analysis by short polyacrylamide gel and modified silver staining. Anal Biochem 365:286–287
20. Greenwood TA, Kelsoe JR (2003) Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene. Genomics 82:511–520
21. Le Hir H, Nott A, Moore MJ (2003) How introns influence and enhance eukaryotic gene expression. Trends Biochem Sci 28: 215–220
22. Van Laere AS, Nguyen M, Braunschweig M, Nezer C, Collette C, Moreau L, Archibald AL, Haley CS, Buys N, Tally M (2003) A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425:832–836
23. Sobrier ML, Maghnie M, Vie-Luton MP, Secco A, di Iorgi N, Lorini R, Amselem S (2006) Novel HESX1 mutations associated with a life-threatening neonatal phenotype, pituitary aplasia, but normally located posterior pituitary and no optic nerve abnormalities. J Clin Endocrinol Metab 91:4528
24. Nackley AG, Shabalina SA, Tchivileva IE, Satterfield K, Korchynskyi O, Makarov SS, Maixner W, Diatchenko L (2006) Human catechol-*O*-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. Science 314:1930
25. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, Gottesman MM (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 315:525