

SNP Identification and Analysis in Part of Intron 2 of Goat *MSTN* Gene and Variation within and among Species

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Part of intron 2 of the myostatin (*MSTN*) gene of 140 goats from 24 populations and 38 sheep from 8 breeds were sequenced, and similar sequences of different species from Gene bank were also obtained to study *MSTN* diversity within and among species. The results indicated that there were seven polymorphic sites in the sequenced region of goat, which have not been separated by recombination (or recurrent mutation), presented complete linkage disequilibrium, and could be sorted into three haplotypes. There was no polymorphic site in the sequenced region of sheep. The haplotype diversity, nucleotide diversity, and average number of single nucleotide polymorphism (SNP) differences of goats from the South group are higher than those of North group, and the corresponding value of the Foreign group is also higher than that of Chinese. The genetic differentiation (0.7558) between the Foreign and Chinese group is significant. There are two main haplotypes of the *MSTN* intron 2 in the goat, which may represent two ancestral types, in support of the theory that domestic goats in the world mainly originated from two ancestors based on morphology, history, archaeology, and molecular markers. The sequence differences of the *MSTN* intron 2 among species are greater than those within species.

The myostatin (*MSTN*) gene has been considered as an important candidate gene for growth and development of domestic animals due to its key role in muscle growth and its potential applications in animal husbandry (Miranda et al. 2002). Goat, as one of the livestock species, has good adaptability, wide geographic distribution, and very abundant breed resources. In China, there are about 24 indigenous goat breeds, distributed in different environmental areas including the north pastoral region, the Qinghai-Tibet plateau region, the mixed pastoral-agricultural region, and the north and south agricultural regions (Compiling Group of Sheep and

Goat Breeds in China 1989), which represent important genetic resources due to their special economic and ecological characteristics. Up to now, the goat *MSTN* gene has been sequenced for intron 1 (Gene Bank Accession No. AF393619), exon 2 (AB077206), intron 2 (AY032689), and exon 3 (AB078013). However, there are few reports on polymorphism of goat *MSTN* gene. In this study, part of intron 2 of *MSTN* gene from some Chinese indigenous goat breeds, imported foreign breeds, and sheep breeds was sequenced and analyzed.

Materials and Methods

Sampling and DNA Extraction

A total of 140 goat samples (kidney, placenta, heart, and blood) from 24 populations were collected, spanning extensive geographic region including 11 provinces and autonomous regions in China. The sequenced individuals from China were divided into South and North groups according to their distribution (Compiling Group of Sheep and Goat Breeds in China 1989). The South group includes Tibetan goat (4), Chengdu Brown goat (5), Nanjiang Brown goat (4), Jianchang Black goat (6), and Chuandong White goat (7) from Sichuan Province; Guizhou White goat (13) and Guizhou Black goat (2) from Guizhou Province; Longlin goat (5) and Duan goat (6) from Guangxi Province; Leizhou goat (7) from Guangdong Province; Matou goat (7) from Hunan Province; and Yichang White goat (11) from Hubei Province. The North group includes Inner Mongolia cashmere goat (6) from Inner Mongolia Autonomous region, Liaoning cashmere goat (9) from Liaoning Province, Taihang Mountain goat (3) and Chengde Polled goat (8) from Hebei Province, Shannan White goat (6) from Shanxi Province, and Jining Grey goat (7) from Shandong Province. The Foreign group includes Angora goat (4), Toggenburg goat (4), Nubian

goat (4), and Saanen goat (4) from Sichuan Province and Boer goat (6) from Hebei Province. Meanwhile, four individuals (blood) of F_1 crossed between Boer goat and Tangshan diary goat were also collected. In addition, 38 sheep samples (kidney) from eight breeds were also collected which were small tail Han sheep (4) from Shandong Province; Dorset sheep (3), Mongolian sheep (5), and Ujumuin sheep (4) from Inner Mongolia Autonomous region; Kazakstan sheep (6) and Altay sheep (5) from Xinjiang Autonomous region; Tong sheep (3) from Shanxi Province; and Tibetan sheep (8) from Sichuan Province. All the samples were from different villages and farms, and owners were questioned in detail in order to avoid close relationships. The samples were collected and stored at -70°C before DNA extraction, which was conducted by phenol extraction method. The corresponding sequences in Gene bank of goat (AY032689), sheep (AF266758), cow (AB076403, AF320998, and AY032689), zebu (AY794986), water buffalo (DQ091762), and horse (AY840554) were used to analyze the variation of *MSTN* gene among species.

Primer Design, Polymerase Chain Reaction Amplification, and Sequencing

The 379-bp fragment of *MSTN* gene was amplified with a pair of primers designed by primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) based on goat *MSTN* sequence (AY032689). The forward and reverse primers were 5'ccctcccttactgtcatcc3' and 5'atcaagcccaaaatctctcc3', respectively, and they were synthesized by Shanghai Sangon Biological Engineering & Technology services Co. Ltd (Shanghai, China). The amplified fragment spanned bases from 1898 to 2276 including part of intron 2 and exon 3.

The polymerase chain reaction (PCR) was carried out in PTC-100TM Programmable Thermal Controller (MJ-Research, Inc., Watertown, MA) with a total volume of 30- μl reaction containing 4 μl (75 ng/ μl) of goat genomic DNA, 3 μl of 10 \times PCR standard reaction buffer, 2.4 μl deoxynucleoside triphosphates (2.5 pmol/L of each deoxynucleotide), 1.2 μl (10 pmol/L) of each forward and reverse primer, 0.3 μl (5 U/ μl) of *Taq* DNA Polymerase (TaKaRa Biotechnology Co. Ltd., Dalian, China), and 17.9 μl of distilled water. After predenaturation for 3 min at 94°C , the PCR profile consisted of a denaturation step at 94°C for 45 s, an annealing step at 64°C for 45 s, and an elongation step at 72°C for 1 min for a total of 34 cycles, followed by a final extension of 10 min at 72°C . The PCR products were run on 1.5% agarose gel including 10mg/ml of ethidium bromide, visualized and photographed with gel automatic photographer under UV light, and sequenced by Bioasia Biological & Technology Co. Ltd. (Beijing, China).

Data Analysis

The *MSTN* nucleotide sequences were aligned using BIOEDIT (version 5.0.9). DNASP (version 4.0) was used to sort haplotypes and analyze the haplotype diversity (Hd, Nei 1987), the average number of nucleotide differences (K, Tajima 1983), the nucleotide diversity (π , Hd, Nei 1987),

Table 1. Genetic differentiation and diversity of goat *MSTN* in different groups

Groups	No.	Hd	K	π	Snn	Nm
South	63	0.0922	0.5530	0.0024	0.5747	10.74
North	28	0.0714	0.4290	0.0018		
Chinese	91	0.0850	0.5100	0.0022	0.7558**	1.39
Foreign	17	0.4706	2.7650	0.0118		
Total	108	0.1233	0.0944	0.0040		

South, North, and Foreign refer to the breeds coming from south, north of China, and imported, respectively. Chinese includes South and North. Total includes Chinese and Foreign. F_1 was not included in computation.

** $P < .01$.

and the linkage disequilibrium test (D' , Lewontin 1964). Genetic differentiation (Snn, Hudson 2000) and gene flow (Nm, Hudson et al. 1992) with the permutation test of 1,000 replicates based on nucleotide sequence information between North and South and Foreign and Chinese were computed using DNASP (version 4.0). To study the variation of *MSTN* gene among species, nucleotide-nucleotide BLAST (BLASTN, <http://www.ncbi.nlm.nih.gov/BLAST/>) was used to search for sequences similar to the goat sequenced region. A phylogeny tree of sequences from different species was constructed using MEGA 3.1 with a Kimura two-parameter nucleotide model and a bootstrap of 1,000 replicates.

Results and Discussion

Polymorphism of Sequenced Region

Only the clearly sequenced region from 140 goat samples, consisting of 236 bp from 1950 to 2185 in part of intron 2 of goat *MSTN* gene (AY032689) was analyzed. Seven polymorphic sites were detected, of which T2124C was a singleton variable site with two variants and A1980G, G1981C, A1982G, G1984T, A2121G, and G2174A were parsimony informative sites with two variants. Most individuals (101) were homozygote, AA, GG, AA, GG, AA, TT, and GG at positions 1980, 1981, 1982, 1984, 2121, 2124, and 2174, respectively, constructing Hap I, AGAGATG, with the frequency of 90.99% (101/111) calculated from homozygotes. Another nine individuals were the opposite homozygote, GG, CC, GG, TT, GG, TT, and AA at corresponding positions except for TT at 2124, constructing Hap II, GCGTGTA, with the frequency of 8.11% (9/111). Only one individual mutated from Hap II at position 2124 constructing Hap III, GCGTGCA, with the frequency of 0.90% (1/111). The other 29 individuals were heterozygotes, AG, GC, AG, GT, AG, TC, and GA. No polymorphic sites were found for the sheep *MSTN* gene in the sequenced region. The linkage disequilibrium test shows that D' between any of two SNPs equals 1, inferring that SNPs found in this region have not been separated by recombination (or recurrent mutation) and presented complete linkage disequilibrium during the evolution history. Therefore, if we don't consider

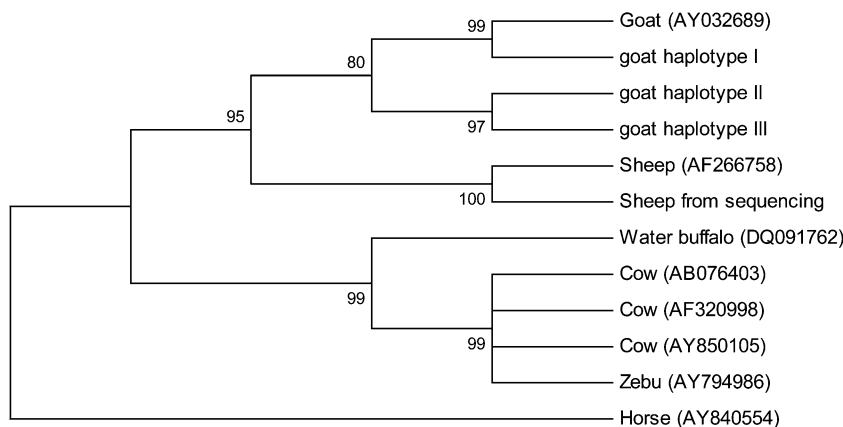


Figure 2. Unweighted Pair Group Method with Arithmetic Mean dendrogram of *MSTN* sequences (236 bp) from different species.

Nucleotide Variation of *MSTN* within and among Species

The number of sequences, Hd, π , and K for North, South, Foreign, and Chinese groups are listed in Table 1. The Snn and Nm from a permutation test of 1,000 replicates from nucleotide sequence-based statistics between North and South and Foreign and Chinese are also shown in Table 1. The Foreign group has the highest Hd (0.4706), K (2.7650), and π (0.0118), which may be related to the fact that individuals in the Foreign group were from different countries and distributed widely. Meanwhile, the corresponding values of South group are higher than those of the North group, indicating that Chinese indigenous goat breeds in south of China have more gene diversity than those in the north of China for *MSTN* gene. This result is consistent with that of RFLP of mtDNA, which indicated that the average π value of South group (0.0516) is higher than 0.0424 of North group (Li et al. 1999) but is not consistent with that of RAPD of genomic DNA (Li et al. 2000) and microsatellite (Li and Alessio 2004), which indicated that there is no obvious difference on genetic diversity between South and North groups. The obviously higher Nm (10.74) and not significant Snn (0.5747) between South and North groups indicated that they do not differentiate significantly and have more gene flow, while the obviously smaller Nm (1.39) and significant Snn (0.7558) between Foreign and Chinese groups indicated that they differentiated significantly and there is little gene flow between them.

The clearly sequenced region (236 bp) of goat representing Hap I was used to search for similar sequences in Gene bank using BLAST. The corresponding sequences in Gene bank of goat (AY032689), sheep (AF266758), cow (AB076403, AF320998, and AY850105), zebu (AY794986), water buffalo (DQ091762), and horse (AY840554) had similarity of 100%, 95%, 93%, 93%, 93%, 93%, 92%, and 80%, respectively, with the goat Hap I. The alignment of sequences from different species is shown in Figure 1. For seven polymorphic sites, one singleton variable site is with two variants at position 2124, five parsimony informative sites are with

two variants at positions 1980, 1982, 1984, 2121, and 2174 (2175 in Figure 1 due to an insertion at position 2134), and one parsimony informative site is with three variants at position 1981. There are many other singleton variable sites and parsimony informative sites with two or three variants in the alignment of sequences from different species.

The goat sequence (AY032689) has the same nucleotides as goat Hap I. Sheep sequences from 38 individuals of eight breeds have the same nucleotides as sheep (AF266758). In comparing three cow sequences, two of them (AF320998 and AY850105) have the same sequences, and cow (AB076403) has an insertion of T at position 2134 and has the same nucleotides as zebu (AY794986). Water buffalo (DQ091762) and horse (AY840554) have less nucleotide similarity than that existing among goat, sheep, and cow. The sequence (236 bp) clustering of different species by MEGA 3.1 with a Kimura two-parameter nucleotide model and a bootstrap of 1,000 replicates was shown in Figure 2. The sequences from within the same species have close relationships and are clustered together. Zebu (AY794986) has a close relationship with cow and next with water buffalo (DQ091762). The horse (AY840554) has the most nucleotide differences compared with other species studied in this paper. The study of genetic diversity of intron 2 of goat *MSTN* gene is generally in agreement with known species relationships and also suggests two major origins of the domestic goat.

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