RESEARCH ARTICLE Indian Journal of Animal Research, Volume 57 Issue 3: 273-281 (March 2023)

Genetic Diversity Identification and Haplotype Distribution of *Myostatin* Gene in Goats

Cheng-Li Liu[#], Ri-Su Na[#], Wei-Wei Ni[#], Guang-Xin E[1], Yan-Guo Han[1], Yan Zeng[1], Xiao Wang[1], Shu-Zhu Cheng[1], Bai-Gao Yang[1], Xing-Hai Duan[1], Ze-Hui Guo[1], Yong-Fu Huang[1]

10.18805/IJAR.B-1305

ABSTRACT

Background: *Myostatin* (*MSTN*) is a highly conserved protein that acts as a negative regulator of skeletal muscle growth. *MSTN* gene is closely associated with multiple biological functions and its mutations are directly linked to muscle development in different species. Loss of *MSTN* functionality causes the phenotype to appear in the form of 'double musculature', among others in cattle, sheep and house mice.

Methods: The mixed DNA pool of Boer and Dazu black goats to sequence *MSTN* coding and noncoding regions. Snapshot typing technology was used to analyse three goat production types, namely, Boer (meat goats), Nubian (breast and meat dual-use goat), Dazu black (local breeds) and Youzhou black goats (local breeds). Polymorphic loci in *MSTN* were used from four goat populations to construct haplotypes and calculate haplotype frequency distribution through NETWORK and MEGA to construct a phylogenetic tree and visualize phylogenetic relationship.

Result: From these populations, 18 haplotypes were constructed using 20 polymorphic loci. Phylogenetic analysis revealed a significant difference in the haplotype distribution of *MSTN* in different goat production types. The 18 haplotypes were divided into two clusters. The haplotypes carried by the Boer goat belonged to Cluster I and those carried by the Nubian goat and two local goat breeds belonged to both clusters. Chinese local goats and complex production goats carried more haplotypes of *MSTN* and had a richer genetic diversity than other production types did. Moreover, local and complex production goats had high-frequency haplotypes of the *MSTN* of meat goats and had high potential for breeding.

Key words: Genetic diversity, Goat, Haplotype, *MSTN*.

INTRODUCTION

MSTN is a member of the TGF-β family and has a highly conserved signal peptide, a TGF-β functional peptide, a hydrolysis site (RARR) and nine strictly conserved cysteins (Liu *et al*., 2016; Huang *et al*., 2016; Hu *et al*., 1998). The core region of TGF-β family members is highly conserved. When mutation causes changes in the core region, the gene's biological activity decreases significantly (Bloise *et al*., 2019; Hu *et al*., 1998). When two cysteine molecules in the activin A core region are replaced with tyrosine, the biological activity and receptor binding of the mutant monomer are only 2% of those of wild-type activin A (Mason *et al*., 1994). When mutations occur in the 175-180 nt region of exon 3 of *MSTN* of mice, the disulphide bond of the mature peptide of *MSTN* is destroyed and the protein structure is consequently changed. The missing protein binding site in the *MSTN* mutant protein affects its ability to bind to the receptor (Chen *et al*., 2019).

MSTN has a role in muscle development. In Belgian Texel sheep, New Zealand Texel sheep, Australian Texel sheep and other types of sheep, the mutation of g.+6723G> A in *MSTN* leads to increased muscle and reduced fat (Haynes *et al*., 2013; Johnson *et al*., 2009; Kijas *et al*., 2007; Clop *et al*., 2006). F94L mutants are found in *MSTN* of Limousin cattle, Angus cattle and a hybrid of these two and the birth weights of these mutant individuals increase by 2.7%, 2.2% and 3.2%, respectively, compared with those of wild-type individuals (Lee *et al*., 2019). F94 sites influence the marbling score, eye muscle area and fat thickness of cattle (Bennett *et al*., 2019). Knocking out *MSTN via* gene editing techniques can lead to skeletal muscle hypertrophy, whereas overexpressing it can cause muscle atrophy (Wang *et al*., 2018; Guo *et al*., 2016; Rodriguez *et al*., 2014). *MSTN* is also a negative regulator inhibiting muscle growth during muscle development and implicated in fat synthesis and glucose metabolism (Zhang *et al*., 2018). Its knockdown in C2C12 myoblasts can diminish lipid formation in myoblasts and regulate the fat content to induce the expression of related genes (Gao *et al*., 2019). After *MSTN* of goats is knocked out, the expression of related genes (SCD and ELOVL6)

[1]College of Animal Science and Technology, Southwest University, Chongqing, China.
[#]These authors are equal contributed.

Corresponding Author: Yong-Fu Huang, College of Animal Science and Technology, Southwest University, Chongqing, China. Email: h67738337@swu.edu.cn

How to cite this article: Liu, C.L., Na, R.S., Ni, W.W., Guang-Xin, E, Han, Y.G., Zeng, Y., Wang, X., Cheng, S.Z., Yang, B.G., Duan, X.H., Guo, Z.H. and Huang, Y.F. (2023). Genetic Diversity Identification and Haplotype Distribution of *Myostatin* Gene in Goats. Indian Journal of Animal Research. 57(3): 273-281. doi: 10.18805/IJAR.B-1305.

Submitted: 12-07-2020 **Accepted:** 30-09-2020 **Online:** 29-12-2020

formed *via* fatty acid metabolism and unsaturated fatty acid biosynthesis changes and through the AMPK signalling pathway to enhance the ratio of AMP/ATP and regulate glucose and lipid metabolism (Xin *et al*., 2020).

Studies on ovine have verified that numerous genetic variations in *MSTN* are significantly associated with growth and carcass traits (Zhang *et al*., 2013). However, the distribution of *MSTN* polymorphic loci in goats with different production types is poorly investigated. In the present study, Boer, Nubian and local Dazu and Youzhou black goats were used to determine the regional genetic variation in *MSTN*. Additionally, the distribution differences in *MSTN* polymorphic loci and their haplotypes were compared in goat populations with different production types.

MATERIALS AND METHODS

In this study, blood samples of 20 goats with no kinship were randomly selected (10 Dazu black goats and 10 Boer goats with equal numbers of males and females) to extract their genomic DNA with phenol-chloroform (Sambrook and Russell 2006). The same amount of DNA from 10 goats from each of the two breeds was mixed with the mixed DNA pool. Nine pairs of amplification primers covering partial regulatory regions, gene coding regions and introns of goats were designed in accordance with the published *MSTN* information (GenBank: JF312048.1 and JX078969.1). Primers were synthesised by Tianyi Huiyuan Biological Technology Company in China and the information of primers is shown in Table 1.

PCR was performed using a PCR kit (Tiangen Biochemical Technology Company, Beijing) for PCR amplification. The reaction system (50 μL) consisted of the following: 5 μL of 10× Taq buffer, 4 μL of 2.5 mM dNTP mixture, 0.5 μL of 2.5 U/μL DNA polymerase, 1 μL of upstream primer F, 1 μL of downstream primer R, 1 μL of DNA and 37.5 μL of ddH_2O. The reaction conditions were as follows: initial denaturation at 95°C for 5 min, denaturation at 95°C for 30 s, annealing for 30 s, extension at 72°C for 1 min, 35 times of recycling, extension at 72°C for 10 min and preservation at 4°C before use. PCR products were detected through agarose gel electrophoresis and sent to Tianyi Huiyuan Company (Wuhan) for sequencing.

A total of 148 female goats (48 Boer goats [Australia Boer goat conservation field, Australia], 40 Nubian goats [Nubian goat conservation field, Yunnan, China], 35 Youzhou black goats [Youyang, China] and 25 Dazu black goats [Dazu, China]) were chosen to determine all the mutation sites in the selected *MSTN*. Nineteen sites of all individuals (the above sequencing results) were typed using SNPshot technology. Sanger technology was employed to process the genotypes in Snp_3710, Snp4728 and 10 mutation sites within snp5426-Snp5620. The primers are shown in Table 3 and the information of PCR primers used in Snapshot technology detection is displayed in Table 2.

Chromas software was utilised to view and edit a Sanger sequencing peak map (Li *et al*., 2003). MEGA5.1 was applied to construct a sequence assembly, sequence alignment and a phylogenetic tree (Dehghan and Yarizade, 2014); DNAsp 5.0 software was used to calculate the number of alleles in mutation sites and construct haplotypes (Librado *et al*., 2009). NETWORK software was employed for haplotype frequency distribution and phylogenetic relationship visualization (Liang *et al*., 2018).

RESULTS AND DISCUSSION

Identification of the gene diversity of *MSTN*

According to the mixed DNA pool sequence, 29 single nucleotide polymorphic sites were identified in the *MSTN*

Table 1: Primer information of the large PCR-amplified fragment of *MSTN*.

Primer names	Forward primer; Reverse primer	TM*(°C)	Amplified region	Length (bp)
MSTN-1	F: 5′-CGCTGGAAAGCTGAGGAA-3′	57	77-877	801 bp
	R: 5′-AGACAAGTGTCGTCGGGATC-3′			
MSTN-2	F: 5′-AATGAATCAGCTCACCCTTG-3′	59	748-1549	802 bp
	R: 5′-GCTCTTTGCCCTCCTCCT-3′			
MSTN-3	F: 5′-GGCTCCTTGGAAGACGA-3′	57	1358-2190	833 bp
	R: 5′-TAGATATGTGCAGACCAGGTACT-3′			
MSTN-4	F: 5′-GGTCTGCACATATCTAGGAGG-3′	55	2175-2857	683 bp
	R: 5′-TACCAATCAGTCTGGAGGAA-3′			
MSTN-5	F: 5′-ATTCCCGAGGCTCAGTT-3′	54	2769-3552	785 bp
	R: 5′-CCTAAGTTGGATTCAGGTTGT-3′			
MSTN-6	F: 5′-GAATCCGATCTCCGAAAC-3′	50	3444-4203	760 bp
	R: 5′-GCATCAGTAGTCTATACAACAAGG-3′			
MSTN-7	F: 5′-GCTTTCCATTGATGTGCTAC-3′	56	3916-4852	937 bp
	R: 5′-GCCATGTCACCAACTTTG-3′			
MSTN-8	F: 5′-ATTACCAGGTCTATTTTGATT-3′	55	4760-5446	687 bp
	R: 5′-GAGCTTTGTCTTGTTTCTTCT-3′			
MSTN-9	F: 5′-TTCCTCCCTGCTCCTTTC-3′	56	5314-6029	716 bp
	R: 5′-ACCCACAGCGATCTACTACC-3′			

Table 2: Primer information of *MSTN* 19 SNPs genotyping by Snapshot in four goat breeds.

Primer names	Conventional primers (5′-3′)	Single base extension primers (5′-3′)
snp-425_F	5′-CAATGAAAACACACATCCTC-3′	5′-AGATTTATTCAGGAAGACTTAAAT-3′
snp-425_R	5′-CAGATTTATTCAGGAAGAC-3′	
snp-1062_F	5′-GGCTTGGCGTTACTCAAAAG-3′	5′-CATAAATAGGTAAATATAAACAAAGATT-3′
snp-1062_R	5′-TCCAGGCGAAGTTTACTGAG-3′	
snp-1226_F	5′-GGATCTGAATGAGAACAGCG-3′	5′-AGAAGCCATAAAAATCCAA-3′
snp-1226_R	5′-GTAACGTGGTAGTCATCGTC-3′	
snp-1583_F	5′-ACGACGGAAACGGTCATTAC-3′	5′-ACTTCATGAGAAATATGCTAAT-3′
snp-1583_R	5′-GAATGCTATGCGAGCATTTC-3′	
snp-2732_F	5′-TGCTAAGTGCTTCTGCTTAC-3′	5′-AAAAGGCCGTCCCAG-3′
snp-2732_R	5′-AGCAGCTGTACCAATCAGTC-3′	
snp-2738_F	5′-TGCTAAGTGCTTCTGCTTAC-3′	5′-GACTCGTCCCAGYGCCCT-3′
Snp-2738_R	5′-AGCAGCTGTACCAATCAGTC-3′	
snp-2752_F	5′-TTCCTTTAAAAGGCCGTCCC-3′	5′-CGGGAATTAGTAGAAAACATAG-3′
snp-2752_R	5′-GACAAGACACAGTGAGCAAC-3′	
snp-3104_F	5′-GGATGTTCTTCCACAGTGTC-3′	5′-TTGTTATACTTGATATTTTGCTG-3′
snp-3104_R	5′-GCCTCCATGTCAATCTATGA-3′	
snp-3456_F	5′-GTAGTAAAGGCCCAACTGTG-3′	5′-ATACTGGAATCCGATCTC-3′
snp-3456_R	5′-TCTGGGAAGGTTACAGCAAG-3′	
snp-3754_F	5′-CAGTGTTGCAAAACTGGCTC-3′	5′-GTACAGGGCTACCATC-3′
snp-3754_R	5′-GGGACACCTACTTTTATTGG-3′	
snp-3819_F	5′-CCTGTGCTTATAAGCCAGAC-3′	5′-ACCCTATGAAAACTCTCTTGA-3′
snp-3819_R	5′-GTGTGTACTAGAATCCACTG-3′	
snp-4036_F	5′-TTTTTTCCCCCAGAAGAGTG-3′	5′-TCTGCTTTACTAAGCAAAC-3′
snp-4036_R	5′-GGTAGTACAAGTTAACTAGCC-3′	
snp-4187_F	5′-TTTTTTCCCCCAGAAGAGTG-3′	5′-CATCAGTGCATCAGTAGTCTATA-3′
snp-4187_R	5′-GGTAGTACAAGTTAACTAGCC-3′	
snp-4443_F	5′-AGACTACTGATGCACTGATG-3′	5′-ACAAGCATAGATAAGCCAG-3′
snp-4443_R	5′-CAGAAGTAAATACAAGCATAG-3′	
snp-4530_F	5′-AGAGCTACTCACTCTTCTGG-3′	5′-ACTTTTTATTTCCATTTATTGATATAAT-3′
snp-4530_R	5′-AGAAAAATTGATCACTTGG-3′	
snp-4552_F	5′-AGAGCTACTCACTCTTCTGG-3′	5′-ATAATWTACAACAAAAGATTATACTTG-3′
snp-4552_R	5′-AGAAAAATTGATCACTTGG-3′	
snp-4609_F	5′-AGAGCTACTCACTCTTCTGG-3′	5′-ACATTTCTTATAGTATAGATTTACTAT-3′
snp-4609_R	5′-GAAAAATTGATCACTTGG-3′	
snp-5167_F	5′-GAAAAGATTGGTGCAGGAGG-3′	5′-GGTGGCAACTATTTTGTTT-3′
snp-5167_R	5′-CATTCTCCCTTCACTTGGTC-3′	
snp-5261_F	5′-ACAAGACCAAGTGAAGGGAG-3′	5′-TTCCTGCTTATTGATTAAATATGGT-3′
snp-5261_R	5′-AAGTCTACAGGAGCCATAGG-3′	

sequence (Table 4). Among them, one single nucleotide mutation site was in the 52 UTR, two were in exon 1, one was in exon 2, five were in intron 1 and the 20 remaining single nucleotides were in intron 2. Twenty disparate sites were found between Dazu and Boer goats. One mutation site was in the regulatory region and the 19 remaining mutation sites were in the intron region.

Haplotype distribution

Statistical analysis revealed that polymorphism appeared in 20 out of 29 mutation sites in all the four goat breeds. About 18-20 SNP sites were detected in Nubian and local Chinese goats and only five SNPs were found in Boer goats (Table 5).

Among the 148 goats, 20 mutation sites constituting 18 haplotypes were found. The haplotypes in different breeds had diverse frequency and distribution. For instance, haplotype H_2 in the four populations accounted for 54.05% of the total number of haplotypes. However, most of the haplotypes did not have high frequencies. Only 1/3 of the haplotypes in one population were unique and the rest had excessively low frequencies. H_18 only appeared in Dazu black goats and its frequency was 0.3%. According to the types of haplotypes in each goat population, the Nubian goats carried the most haplotypes of up to 15. Among them, H_9, H_12, H_13 and H_14 were unique and constituted less than 1% of the total sample in Nubian goats. Dazu black

Table 3: Primer information of 10 SNPs of *MSTN* genotyped *via* Sanger sequencing in four goat breeds.

Primer names	Conventional primers (5′-3′)
Snp_3710_F	5′-GAATCCGATCTCCGAAAC-3′
Snp_3710_R	5′-GCATCAGTAGTCTATACAACAAGG-3′
Snp_4728_F	5′-GCTTTCCATTGATGTGCTAC-3′
Snp_4728_R	5′-GCCATGTCACCAACTTTG-3′
Snp_5426-Snp_5620_F	5′-CTTCTTTCCTCCCTGCTCCT-3′
Snp_5426-Snp_5620_R	5′-CCCATCCAAAAGCTTCAAAA-3′

Table 4: 29 SNPs in the full- length DNA mixed pool sequence of *MSTN* of Boer and Dazu black goats.

Position	Locus (bp)	Template	Boer goats	Dazu black goats
5′UTR	425	C	C	C/T
Exon 1	1062	G	A	A
Exon 1	1226	G	A	A
Intron 1	1583	A	A/G	A
Intron 1	2732	C	C/T	C/T
Intron 1	2738	G	A	A
Intron 1	2752	G	A/G	A/G
Intron 1	3104	G	T	T
Exon 2	3456	C	T	T
Intron 2	3710	C	C	C/T
Intron 2	3754	A	C	C
Intron 2	3819	C	C	C/T
Intron 2	4036	T	T	C/T
Intron 2	4187	G	G	G/T
Intron 2	4443	T	C	C/T
Intron 2	4530	T	T	A/T
Intron 2	4552	A	A/C	A/C
Intron 2	4609	T	T	C/T
Intron 2	4728	A	A	A/G
Intron 2	5167	C	C	C/T
Intron 2	5261	A	C	C
Intron 2	5426	A	A	A/G
Intron 2	5427	G	G	C/G
Intron 2	5428	A	A	A/G
Intron 2	5430	G	G	G/T
Intron 2	5502	C	T	T
Intron 2	5567	A	A	A/G
Intron 2	5600	T	C	C
Intron 2	5620	G	G	A/G

goats possessed eight haplotypes among which H_17 and H_18 were unique with frequencies of 1.0% and 0.3%, respectively. Boer goats had six haplotypes; among them, H_3 only appeared in this breed and accounted for 1% of the total number. Youzhou black goats had five haplotypes and no unique haplotype (Table 6).

Haplotype network and phylogeny clustering distribution maps revealed that the 18 haplotypes could be divided into two clusters (Best Fitting Model, Table S1). Cluster I included 10 haplotypes, namely, H_1, H_2, H_3, H_4, H_5, H_6, H_10, H_11, H_1 and H_15. Cluster II included eight haplotypes, namely, H_7, H_8, H_9, H_12, H_13, H_16, H_17 and H_18. According to their distribution, the haplotypes in Boer goats were classified as Cluster I and those carried by Nubian goats, Dazu black goats and Youzhou black sheep belonged to both clusters (Fig 1).

Table 5: Mutation type of 20 SNPs of *MSTN* in four goat breeds.

SNPs	Boer goats	Nubian goats	Dazu black goats	Youzhou black goats
Snp_425		C/T	C/T	C/T
Snp_1583	A/G	A/G	A/G	
Snp_2732	C/T	C/T	C/T	C/T
Snp_2738	A/G	A/G	A/G	
Snp_2752	A/G	A/G	A/G	A/G
Snp_3710		C/T	C/T	C/T
Snp_3819		C/T	C/T	C/T
Snp_4036		C/T	C/T	C/T
Snp_4187		G/T	G/T	G/T
Snp_4443		C/T	C/T	C/T
Snp_4530		A/T	A/T	A/T
Snp_4552	A/C	A/C	A/C	A/C
Snp_4728		A/G	A/G	A/G
Snp_5167		C/T	C/T	C/T
Snp_5426		A/G	A/G	A/G
Snp_5427		C/G	C/G	C/G
Snp_5428		A/G	A/G	A/G
Snp_5430		G/T	G/T	G/T
Snp_5567		A/G	A/G	A/G
Snp_5620		A/G	A/G	A/G
Total	5	20	20	18

The genetic diversity of *MSTN* in goats has been widely studied. However, the polymorphism of *MSTN* varies in different goat populations. Li *et al.* (2006a) detected seven polymorphic loci in the intron 2 of 24 goat populations. Liu (2006) discovered eight polymorphic loci in Chinese local breeds. One polymorphic locus is in 5′UTR, one is in exon 1 and six are in intron 2. Li *et al.* (2006b) found eight SNPs in intron 2 and exon 3 of 22 goat breeds and they include seven SNPs in intron 2 and one SNP in exon 3. Some scholars revealed only 1 to 2 polymorphic sites in exon 3 of local and commercial goat breeds here and abroad (Khichar *et al.*, 2016; Min *et al.*, 2015; Wang *et al.,* 2008). All these reports are in accordance with the present findings. Most polymorphic loci are assembled in intron 2. Other sequences, especially in exon 2, where no mutation sites have been found, are rarely mutated. This finding indicated differences in the conservative property of diverse *MSTN* regions. With respect to the total number of SNPs, the number of SNPs in *MSTN* of Nubian goats, Youzhou black goats and Dazu black goats is higher than that of Boer goats and others. These three breeds had rich genetic diversity and hence had a high breeding value.

Table 6: Comparison of the *MSTN* haplotype frequency of four goat breeds.

Haplotypes	Sequence of haplotype	Boer goats	Nubian goats	Dazu black goats	Youzhou black goats	Total	Proportion of haplotype (%)
H_1	CGTAACCTGCTCACAGAGAG	22	9	0	0	31	10.5
H_2	CACAGCCTGCTAACAGAGAG	59	31	24	46	160	54.1
H_3	CACGACCTGCTAACAGAGAG	3	0	0	0	3	1.0
H_4	CGTAGCCTGCTAACAGAGAG	6	1	0	0	7	2.4
H_5	CATAGCCTGCTAACAGAGAG	5	3	0	0	8	2.7
H_6	CATAACCTGCTAACAGAGAG	1	2	0	0	3	1.0
H_7	CATAATCCTTACGCGCGTGA	0	2	0	1	3	1.0
H_8	CGTAATTCTTACGCGCGTGA	0	8	1	0	9	3.0
H_9	CATAATTCTTACGCGCGTGA	0	2	0	0	2	0.7
H_10	CATAACCTGCTCACAGAGAG	0	11	5	3	19	6.4
H_11	CACAGCCTGCTAATAGAGAG	0	2	3	1	6	2.0
H_12	CGTAATTCTTACGCGCGTGG	0	2	0	0	2	0.7
H_13	CGTAACTCTTACGCGCGTGA	0	2	0	0	2	0.7
H_14	CATAACCTGCACACAGAGAG	0	2	0	0	2	0.7
H_15	CACGGCCTGCTAACAGAGAG	0	1	1	0	2	0.7
H_16	TATAATTCTTACGCGCGTGA	0	2	13	18	33	11.1
H_17	TATAATTCTTACGCGCGTGG	0	0	3	0	3	1.0
H_18	TATAACTCTTACGCGCGTGA	0	0	1	0	1	0.3

Note: The base positions and mutation types of each haplotype (Table 8) are 425 (C/T), 1583 (A/G), 2732 (C/T), 2738 (A/G), 2752 (A/G), 3710 (C/T), 3819 (C/T), 4036 (C/T), 4187 (G/T), 4443 (C/T), 4530 (A/T), 4552 (A/C), 4728 (A/G), 5167 (C/T), 5426 (A/G), 5427 (C/G), 5428 (A/G), 5430 (G/T), 5567 (A/G) and 5620 (A/G).

Table 7: Estimation of the best fitting model of the phylogenetic relationship in 18 haplotypes.

Model	Parameters	BIC	AICc	lnL	Invariant	Gamma
K2+G	35	462.734	334.498	-128.36	n/a	59.28
K2	34	463.931	339.126	-131.902	n/a	n/a
K2+G+I	36	468.62	336.968	-128.36	0	59.28
T92	35	469.203	340.967	-131.595	n/a	n/a
K2+I	35	469.817	341.581	-131.902	0	n/a
T92+G	36	474.96	343.308	-131.53	n/a	10.52
T92+I	36	475.048	343.396	-131.574	0	n/a
TN93	38	478.915	340.477	-127.622	n/a	n/a
HKY	37	480.189	345.136	-131.201	n/a	n/a
JC	33	480.452	359.094	-143.105	n/a	n/a
T92+G+I	37	480.846	345.793	-131.53	0	10.52
TN93+G	39	484.516	342.708	-127.479	n/a	28.75
HKY+G	38	484.635	346.196	-130.481	n/a	10.11
HKY+I	38	485.842	347.404	-131.085	0	n/a
JC+I	34	486.34	361.535	-143.106	0	n/a
JC+G	34	488.866	364.061	-144.369	n/a	200
TN93+G+I	40	490.402	345.24	-127.479	0	28.75
HKY+G+I	39	490.521	348.713	-130.481	0	10.11
TN93+I	39	491.842	350.034	-131.142	0	n/a
JC+G+I	35	494.752	366.516	-144.369	0	200
GTR	41	494.836	346.336	-126.753	n/a	n/a
GTR+G	42	506.727	354.905	-129.755	n/a	11.87
GTR+I	42	506.877	355.055	-129.83	0	n/a
GTR+G+I	43	512.613	357.485	-129.755	0	11.87

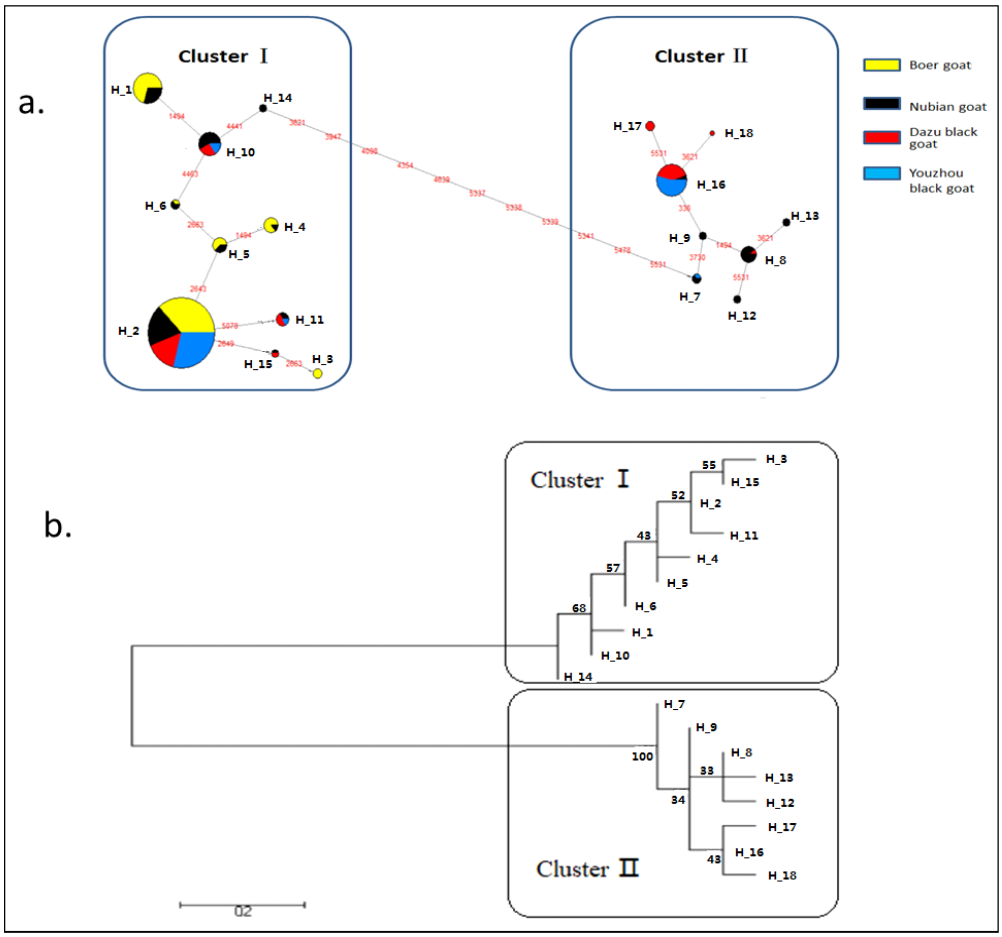

Fig 1: (a) Median joining network of 18 haplotypes of *MSTN* from four goat breed (n=148) samples, based on DNA sequences (*MSTN*); (b) maximum-likelihood (ML) phylogenetic tree of *MSTN* constructed with MEGA 5.0 by using the sequences of *MSTN* from four goat breeds, regions (n=148) in this study and the sequences available in GenBank. The scale bar represented the inferred substitutions per nucleotide site. The relative supports of the clades in the tree produced from the ML analyses were indicated above and below the branches, respectively.

MSTN gene polymorphic loci play an important role in the growth of goats. Mutations in nucleotides at different positions in *MSTN* sequence result in disparate characters in goats. For instance, a polymorphic locus detected in the *MSTN* gene of Boer and Anhui white goats is associated with goat weight at 12 months of age, body length and height at withers (Zhang *et al.,* 2013). For the T/C mutation at base 3783 in intron 2 of *MSTN* of Haimen goats, its polymorphism substantially influences the goat's birth weight (p<0.01; Zhang 2009). Furthermore, the function of the same polymorphic locus differs in various goat populations. The insertion and deletion of 5 bp TTTTA in the 5′UTR of *MSTN* are observed in Boer goats, Inner Mongolia White Cashmere goats and Shaanbei White Cashmere Goat and this mutation site is prominently relevant to the growth of goats, especially to the chest depth, height and chest circumference of Inner Mongolia White Cashmere goats and Shaanbei White Cashmere goats. However, studies have yet to determine if the site has the same genetic effect in other populations (Bi *et al*., 2020a; Bi *et al*., 2020b; Zhang *et al*., 2012). These studies have illustrated that *MSTN* plays an important role in the growth and development of goats and is inseparable from the multifunctional polymorphic sites crucial in goat breeding. Additionally, *MSTN* has abundant genetic diversity, which is important in breeding various types of goats.

A large number of *MSTN* gene polymorphic loci functioning in goat growth should be explored in detail. However, polymorphic loci follow the general distribution pattern after haplotypes in Boer goats, Nubian goats, Youzhou black goats and Dazu black goats are constructed. The haplotype distribution in Boer goats is concentrated, whereas the distribution in the three other goat breeds is highly scattered. A similar situation is observed in sheep. Fifteen SNP sites with 12 haplotypes are detected in *MSTN* of local Chinese sheep breeds (9) and imported sheep breed. Haplotype VIII is a haploid homozygous in all-meat or meat/wool sheep. Haplotype I is only distributed in skin type and skin/meat type goat breeds (Gong *et al*., 2009). Polymorphic loci are discovered in *MSTN* of Yanqi horse and Yili horse and they have three genotypes: AA, AG and

Table 8: Nucleotide composition of 18 haplotypes in 20 SNPs.

Haplotypes	425	1583	2732	2738	2752	3710	3819	4036	4187	4443	4530	4552	4728	5167	5426	5427	5428	5430	5567	5620
H_1	C	G	T	A	A	C	C	T	G	C	T	C	A	C	A	G	A	G	A	G
H_2	C	A	C	A	G	C	C	T	G	C	T	A	A	C	A	G	A	G	A	G
H_3	C	A	C	G	A	C	C	T	G	C	T	A	A	C	A	G	A	G	A	G
H_4	C	G	T	A	G	C	C	T	G	C	T	A	A	C	A	G	A	G	A	G
H_5	C	A	T	A	G	C	C	T	G	C	T	A	A	C	A	G	A	G	A	G
H_6	C	A	T	A	A	C	C	T	G	C	T	A	A	C	A	G	A	G	A	G
H_7	C	A	T	A	A	C	C	T	T	T	A	C	G	C	G	C	G	T	G	A
H_8	C	G	T	A	A	C	T	C	T	T	A	C	G	C	G	C	G	T	G	A
H_9	C	A	T	A	A	T	T	C	T	T	A	C	G	C	G	C	G	T	G	A
H_10	C	A	T	A	A	C	C	T	G	C	T	C	A	C	A	G	A	G	A	G
H_11	C	A	C	A	G	T	C	T	G	C	T	A	A	T	A	G	A	G	A	G
H_12	C	G	T	A	A	T	T	C	T	T	A	C	G	C	G	C	G	T	G	G
H_13	C	G	T	A	A	C	T	C	T	T	A	C	G	C	G	C	G	T	G	A
H_14	C	A	T	A	A	C	C	T	G	C	A	C	A	C	A	G	A	G	A	G
H_15	C	A	C	G	G	C	C	T	G	C	T	A	A	C	A	G	A	G	A	G
H_16	T	A	T	A	A	T	T	C	T	T	A	C	G	C	G	C	G	T	G	A
H_17	T	A	T	A	A	T	T	C	T	T	A	C	G	C	G	C	G	T	G	G
H_18	T	A	T	A	A	C	T	C	T	T	A	C	G	C	G	C	G	T	G	A

GG. This site deviates from the Hardy-Weinberg equilibrium. The correlation analysis of different genotypes and body size traits shows that the chest circumference of GG and AG types of Yanqi horse is significantly longer than that of AA type ($p<0.05$). The body length of the GG-type Yili horse is longer than that of AA type. The chest circumference of the GG type is remarkably longer than that of the AG and AA types (Han 2015). Meanwhile, 11 polymorphic loci with 12 haplotypes are found in *MSTN* of commercial and local breed chickens. Haplotypes are closely related to the body weight of chicken on days 1, 14, 28 and 42 and carcass traits on day 42 (Dushyanth *et al*., 2016). The ~622 bp A/T mutation in the 5′UTR of *MSTN* is associated with the economic traits of Boer goats and three other local goats (Lubei White, Laoshan Dairy and Wendeng Dairy goats). The CC genotype is primarily concentrated in the Boer goat population and TT is mainly concentrated in the three local populations; the weaning weight and net meat weight of the CC-type individuals are significantly higher than those of TT types (Min *et al*., 2015).

Different genotypes or haplotypes have disparate distributions in animal populations with different production types. This result implies that a dominant genotype is gradually achieved. This finding is consistent with the breeding expectations of long-term artificial selection. Boer goat is the best meat goat breed in the world and its haplotypes related to economic traits accumulate through long-term artificial selection. As local indigenous goat breeds, Dazu and Youzhou black goats are bred through natural selection and environmental adaptability without powerful manual selection. The lactation and meat performance of Nubian goats, as a milk/meat dual type, are achieved through artificial breeding (Kholif *et al*., 2018). However, the emphasis on production speed and meat production is weaker than the selection pressure of Boer goats because they are bred for their meat and milk. As a result, the genetic diversity of the selected genomic regions related to traits varies.

CONCLUSION

In this research, the genetic diversity of a large number of *MSTN* was identified and an in-depth understanding of the conservative property and polymorphism of the coding and noncoding sequences of *MSTN* was provided. This study revealed that the haplotypes related to economic traits were concentrated in meat goat breeds by employing different goat populations of disparate production types and analysing the haplotype of *MSTN*. The haplotypes in local and breast/meat dual-use goat breeds were rarely selected and their *MSTN* had a high genetic diversity. Their haplotypes included those of meat goats, which showed potential for further breeding to some extent. This study provided a reference for future research on the function of *MSTN*, the development of effective breeding markers in goat breeding and *MSTN* to improve valuable production traits after mutations.

ACKNOWLEDGEMENT

This work was supported by the Characteristic Germplasm Resources Population Selection and Innovation on Mutton Sheep and Goats (No. 2015BAD03B05), National Natural Science Foundation of China (No. 31172195), Chongqing Research Program of Basic Research and Frontier Technology (cstc2018jcyjAX0153) and Fundamental Research Funds for the Central Universities (XDJK2018B014 and XDJK2017A003).

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Bennett, G.L., Tait, R.G.J., Shackelford, S.D., Wheeler, T.L., King, D.A., Casas, E. and Smith, T.P.L. (2019). Enhanced estimates of carcass and meat quality effects for poly--morphisms in myostatin and μ-calpain genes. J. Anim. Sci. 97(2): 569-577.

Bi, Y., He, L., Feng, B., Lan, X., Song, X., Qu, L. and Pan, C. (2020a). A 5-bp mutation within *MSTN*/GDF8 gene was significantly associated with growth traits in Inner Mongolia White Cashmere goats. Anim Biotechnol. 32(2): 1-6.

Bi, Y., Feng, B., Wang, Z., Zhu, H., Qu, L., Lan, X., Pan, C. and Song, X. (2020b). Myostatin (*MSTN*) gene indel variation and its associations with body traits in Shaanbei White Cashmere goat. Animals (Basel). 10(1): 168. doi: 10.3390/ani10010168.

Bloise, E., Ciarmela, P., Dela, Cruz, C., Luisi, S., Petraglia, F. and Reis, FM. (2019). Activin A in mammalian physiology. Physiol. 99(1): 739-780.

Chen, C., Zhu, L., Zhou, X., Bai, C., Zheng, C., Wei, Z. (2019). The effect of mice (*mus musculus*) myostatin Y309 and C310 deletions on protein structure. Journal of Agricultural Biotechnology. (6): 1051-1061.

Clop, A., Marcq, F., Takeda, H., Pirottin, D., Tordoir, X., Bibé, B., Bouix, J., Caiment, F., Elsen, J.M., Eychenne, F., Larzul, C., Laville, E., Meish, F., Milenkovic, D., Tobin, J., Charlier, C. and Georges, M. (2006). A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. Nature Genetics. 38(7): 813-818.

Dehghan and Yarizade K. (2014). Bioinformatics study of delta-12 fatty acid desaturase 2 (FAD2) gene in oilseeds. Molecular Biology Reports. 41(8): 5077-5087.

Dushyanth, K., Bhattacharya, T.K., Shukla, R., Chatterjee, R.N., Sitaramamma, T., Paswan, C. and Guru Vishnu, P. (2016). Gene expression and polymorphism of myostatin gene and its association with growth traits in chicken. Anim. Biotechnol. 27(4): 269-277.

Gao, L., Yang, M., Wang, X., Yang, L., Bai, C. and Li, G. (2019). *MSTN* knockdown decreases the trans-differentiation from myocytes to adipocytes by reducing *Jmjd3* expression *via* the SMAD2/SMAD3 complex. Biosci Biotechnol Biochem. 83(11): 2090-2096.

Gong, Y.F., Li, X.L., Liu, Z.Z., Jin, X.M., Zhou, R.Y., Li, L.H. and Zhang, Q. (2009). SNP detection and haplotype analysis in partial sequence of *MSTN* gene in sheep. Russ. J. Genet. 45(12): 1454-1457.

Guo, R., Wan, Y., Xu, D., Cui, L., Deng, M., Zhang, G., Jia, R., Zhou, W., Wang, Z., Deng, K., Huang, M., Wang, F. and Zhang, Y. (2016). Generation and evaluation of Myostatin knock-out rabbits and goats using CRISPR/Cas9 system. SCI REP-UK. 6(1): doi: 10.1038/srep29855.

Han, Y.R. (2015). The Polymorphism of *MSTN*, GH Gene and Their Associations with Body Size Traits in Three Horse Breeds. Xinjiang Agricultural University.

Haynes, F.E., Greenwood, P.L., McDonagh, M.B., McMahon, C.D., Nicholas, G.D., Berry, C.J. and Oddy, V.H. (2013). Lack of association between allelic status and myostatin content in lambs with the myostatin g+6723G/A allele. J. Anim Sci. 91(1): 78-89.

Hu, P.P., Datto, M.B. and Wang, X.F. (1998). Molecular mechanisms of transforming growth factor-beta signaling. Endocr. Rev. 19(3): 349-363.

Huang, T. and Hinck, A.P. (2016). Production, isolation and structural analysis of ligands and receptors of the TGF-β super family. Methods Mol. Biol. 1344: 63-92.

Johnson, P.L., Dodds, K.G., Bain, W.E., Greer, G.J., McLean, N.J., McLaren, R.J. Galloway, S.M., van Stijn, T.C. and McEwan, J.C. (2009). Investigations into the GDF8 g+6723G-A polymorphism in New Zealand Texel sheep. Journal of Animal Science. 87(6): 1856-1864.

Khichar, J.P., Gahlot, G.C., Agrawal, V.K. and Kiran Ashraf, M. (2016). Molecular characterization of exon 3 of caprine myostatin gene in Marwari goat. Veterinary World. 9(6): 676-679.

Kholif, A.E., Gouda, G.A., Olafadehan, O.A. and Abdo, M.M. (2018). Effects of replacement of moringa oleifera for berseem clover in the diets of Nubian goats on feed utilisation and milk yield, composition and fatty acid profile. Animal. 12(5): 964-972.

Kijas, J.W., McCulloch, R., Edwards, J.E., Oddy, V.H., Lee, S.H. and van der Werf, J. (2007). Evidence for multiple alleles effecting muscling and fatness at the Ovine GDF8 locus. BMC Genetics. 8(1): 80. doi: 10.1186/1471-2156-8-80.

Lee J., Kim, J.M. and Garrick, D.J. (2019). Increasing the accuracy of genomic prediction in pure-bred Limousin beef cattle by including cross-bred Limousin data and accounting for an F94L variant in *MSTN*. Anim Genet. 50(6): 621-633.

Li, L.L., Lin, Y., Liu, Y., Zeng, E.L. and Li, C.Z. (2003). The use of shareware for mtDNA sequence comparison. Chin. J. Foren. Sci. 1: 29-31.

Li, X.L., Wu, Z.L., Liu, Z.Z., Gong, Y.F., Zhou, R.Y. and Zheng, G.R. (2006a). SNP identification and analysis in part of intron 2 of goat *MSTN* gene and variation within and among species. J. Hered. 97(3): 285-289.

Li, X.L., Wu, Z.L., Gong, Y.F., Liu, Y.Q., Liu, Z.Z., Wang, X.J., Xin, T.R. and Ji, Q. (2006b). Single-nucleotide polymorphism identification in the caprine myostatin gene. J. Anim. Breed Genet. 123(2): 141-144.

Liang, R.Y., Chen, X.Y., Sun, H.X., Sun, S.H., Guo, W.T. and Shen, Z.Q. (2018). Phylogenety and genetic diversity of five local sheep breeds on mtDNA D-loop. Acta Agriculturae Boreali-Sinica.

Librado, P., Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics. 25 (11): 1451-1452.

Liu, C.Z. (2006). The Relationship Between *MSTN* Gene Polymorphism and Perfomance Trait of Goat. Inner Mongolia Agricultural University.

Liu, L., Li, Y.L., Xu, S.D., Wang, K.Z., Wu, P., Chu, W.Y. and Wang, X.Q. (2016). Molecular characterization of the myosatin gene and the effect of fasting on its expression in Chinese perch (*Siniperca chuatsi*). Genet. Mol. Res. 15(2). doi: 10.4238/gmr.15028354.

Mason, A.J. (1994). Functional analysis of the cysteine residues of activin A. Mol. Endocrinol. 8(3): 325-332.

Min, L.J., Feng, Y.N., Li, L. and Li, M.Y. (2015). Associations of *MSTN* gene's polymorphisms with some economic traits in goats. Chinese Journal of Animal and Veterinary Sciences. 46(09): 1515-1524.

Rodriguez, J., Vernus, B., Chelh, I., Cassar-Malek, I., Gabillard, J.C., Hadj Sassi, A., Seiliez, I., Picard, B. and Bonnieu, A. (2014). Myostatin and the skeletal muscle atrophy and hypertrophy signaling pathways. Cell Mol. Life Sci. 71(22): 4361-471.

Sambrook, J. and Russell, DW. (2006). Amplification of cDNA generated by reverse transcription of mRNA. 2006(1).

Wang, L.X. (2008). Genetic Analysis of GH, *MSTN* and GHR Genes and Their Associations with Growth Traits in Goats. Northwest A&F University.

Wang, X., Niu, Y., Zhou, J., Zhu, H., Ma, B., Yu, H., Hua, J., Huang, X., Qu, L. and Chen, Y. (2018). CRISPR/Cas9-mediated *MSTN* disruption and heritable mutagenesis in goats causes increased body mass. Anim. Genet. 49(1): 43-51.

Xin, X., Yang, S., Li, X., Liu, X., Zhang, L., Ding, X., Zhang, S., Li, G. and Guo, H. (2020). Proteomics insights into the effects of *MSTN* on muscle glucose and lipid metabolism in genetically edited cattle. Gen. Comp. Endocrinol. 291: 113237. doi: 10.1016/j.ygcen.2019.113237.

Zhang, C., Liu, Y., Xu, D., Wen, Q., Li, X. and Zhang, W. (2012). Polymorphisms of myostatin gene (*MSTN*) in four goat breeds and their effects on Boer goat growth performance. Molecular Biology Reports. 39(3): 3081-3087.

Zhang, L. (2009). Associations Between Genetic Polymorphisms at Four Loci and Related Traits in Haimen Goats. Yangzhou University.

Zhang, ZJ., Ling, Y.H., Wang, L.J., Hang, Y.F., Guo, X.F., Zhang, Y.H., Ding, J.P. and Zhang, X.R. (2013). Polymorphisms of the myostatin gene (MSTN) and its relationship with growth traits in goat breeds. Genet Mol. Res. 12(2): 965-971.