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Single nucleotide polymorphisms of myostatin gene in Chinese domestic horses

Ran Li [a,1], Dong-Hua Liu [b,1], Chun-Na Cao [c], Shao-Qiang Wang [a], Rui-Hua Dang [a], Xian-Yong Lan [a], Hong Chen [a], Tao Zhang [d], Wu-Jun Liu [e], Chu-Zhao Lei [a,*]

[a] Shaanxi Key Laboratory of Molecular Biology for Agriculture, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] Institute of Hexi Ecology and Oasis Agriculture, Hexi University, Zhangye, Gansu 734000, China
[c] Animal Husbandry and Veterinary Bureau of Yining, Yili, Xinjiang 835100, China
[d] Shaanxi University of Technology, Hanzhong, Shaanxi 723001, China
[e] Xinjiang Agricultural University, Urumqi, Xinjiang 830052, China

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ABSTRACT

The myostatin gene (*MSTN*) is a genetic determinant of skeletal muscle growth. Single nucleotide polymorphisms (SNP) in *MSTN* are of importance due to their strong associations with horse racing performances. In this study, we screened the SNPs in *MSTN* gene in 514 horses from 15 Chinese horse breeds. Six SNPs (g.26 T > C, g.156 T > C, g.587A > G, g.598C > T, g.1485C > T, g.2115A > G) in *MSTN* gene were detected by sequencing and genotyped using PCR-RFLP method. The g.587A > G and g.598C > T residing in the 5′UTR region were novel SNPs identified by this study. The g.2115A > G which have previously been associated with racing performances were present in Chinese horse breeds, providing valuable genetic information for evaluating the potential racing performances in Chinese domestic breeds. The six SNPs together defined thirteen haplotypes, demonstrating abundant haplotype diversities in Chinese horses. Most of the haplotypes were shared among different breeds with no haplotype restricted to a specific region or a single horse breed. AMOVA analysis indicated that most of the genetic variance was attributable to differences among individuals without any significant contribution by the four geographical groups. This study will provide fundamental and instrumental genetic information for evaluating the potential racing performances of Chinese horse breeds.

1. Introduction

The myostatin gene (*MSTN*) belonging to the TGF-β superfamily is widely known for its genetic effect on double muscling trait as a negative regulator of skeletal muscle growth (Grobet et al., 1998). It controls the proliferation of muscle precursor cells by inhibiting cell cycle progression mediated by the p21 gene (Thomas et al., 2000) and participates in bone formation and regeneration by inhibiting the recruitment and proliferation of progenitor cells in the fracture blastema (Hamrick et al., 2010; Kellum et al., 2009). Mutations in the *MSTN* gene have been found to be strongly associated with growth, reproduction and carcass quality traits in many mammalian species. In cattle, six different loss-of-function mutations in the MSTN gene caused an increase in the number of muscle mass resulting in enlarged muscles (Kambadur et al., 1997; Marchitelli et al., 2003). In sheep, mutations in coding region were shown to affect carcass conformation and fatness (Boman and Våge, 2009; Boman et al., 2009, 2010). In dogs, a mutation in the third exon increased muscle mass and enhances racing performance (Mosher et al., 2007).

The equine *MSTN* gene mapped at the distal end of the equine chromosome 18 (ECA18) consists of three exons and two introns (GQ183900.1). In total, 23 equine *MSTN* gene SNPs have been reported by far, namely two in the promoter region, eight in intron-1, one in intron-2, 10 in exon-2 and two in 3′-UTR (Boman et al., 2010; Dall'Olio et al., 2010; Hill et al., 2010). A mutation in intron-1 of the *MSTN* gene can serve as a powerful marker for prediction of race distance aptitude in Thoroughbreds (Hill et al., 2010). The association between *MSTN* gene and horse racing performances was further evidenced by Binns et al. (2010) and Tozaki et al. (2010). In addition, two mutations located in the promoter of the *MSTN* gene are associated with breeds of different morphological types (Dall'Olio et al., 2010) while eight mutations in exon-2 caused alterations of protein function (Baron et al., 2012).

In Chinese horse breeds, sequence variants of *MSTN* gene have been poorly characterized with only limited SNPs identified in Mongolian

Abbreviations: MSTN, myostatin gene; SNP, single nucleotide polymorphism; RFLP, restriction fragment length polymorphism; TGF-β, transforming growth factor beta; p21, cyclin-dependent kinase inhibitor 1 encoded by the CDKN1A gene; 3′-UTR, 3′ untranslated region; 5′-UTR, 5′ untranslated region; ACD, acid citrate dextrose; Hd, haplotype diversity; AMOVA, analysis of molecular variance; HWE, Hardy–Weinberg equilibrium.

* Corresponding author at: College of Animal Science and Technology, Northwest A & F University, Yangling, Shaanxi 712100, China. Tel.: +86 29 87091373; fax: +86 29 87092164.
E-mail address: leichuzhao1118@126.com (C.-Z. Lei).

[1] The two authors contributed equally to this article.

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Table 1
Sampling information of the Chinese domestic horse breeds in this study.

Distribution	Breed	Abbreviation	Sample size	Source region
Northwestern China	Kazakh	HSK	20	Changji, Yili county, Xinjiang
	Yanqi	YQ	42	Hejing county, Xinjiang
	Balikun	BLK	24	Balikun country, Xinjiang
	Chakouyi	CKY	50	Tianzhu county, Gansu
	Datong	DT	32	Qilian county,Qinghai
	Yushu	YS	56	Yushu county,Qinghai
	Hequ	HQ	30	Maqu county, Gansu
	Chaidamu	CD	42	Geermu city, Qinhai
	Ningqiang	NQ	29	Ningqiang county, Shaanxi
	Guanzhong	GU	28	Fufeng county, Shaanxi
Northeastern China	Mongolian	MG	16	Inner Mongolia
Central China	Lichuan	LC	21	Lichuan city, Hubei
Southwestern China	Guizhou	GZ	49	Guiyang city, Guizhou
	Debao	DB	39	Debao county, Guangxi
	Baise	BS	36	Baise county, Guangxi

horse (Wang, 2005). The significance of *MSTN* SNPs on horse racing performance urges the need for a thorough characterization of *MSTN* polymorphisms among Chinese domestic horses. Here the partial sequence of the *MSTN* gene including 5′-UTR region (671 bp), intron-1 (1829 bp), exon-1 (373 bp) and exon-2 (374 bp) in 15 Chinese horse breeds was screened. Our aim is to screen the sequence polymorphisms of the *MSTN* gene and analyze their genetic diversities in Chinese horse breeds. Considering the strong association between *MSTN* gene and racing performance, this study will provide instrumental genetic information for evaluating the potential racing performances of Chinese horse breeds and helps to select the best Chinese domestic breed for further cultivation towards optimum racing traits.

2. Methods and materials

2.1. Specimen collection and DNA extraction

A total of 514 blood samples from 15 Chinese native horse breeds were divided into four groups based on their geographical distributions (Northwestern, Northeastern, Central and Southern China). The Guanzhong horse (GU) belongs to the cultivated breed. Details of the horse breeds involved in the analysis are presented in Table 1. All the samples were collected randomly from local villages based on the pedigree information from the herdsmen to prevent the related individuals and ensure the coverage of native tract and purity of each population. 20 ml of jugular blood was collected for each sample, immediately treated by 4 ml of ACD anti-coagulation and stored at −4 °C for transportation. Genomic DNA was extracted from blood using Genomic DNA isolation kit (Sangon, Shanghai, China) according to the manufacturer's instructions.

2.2. PCR amplification

In total, 3267 bp of DNA fragments (GQ183900.1) was amplified including partial 5′-UTR (671 bp), exon-1 (373 bp), intron-1 (1829 bp), and exon-2 (374 bp) of the equine MSTN gene. Primers (Table 2) were designed using Primer Premier 5.0 software (PREMIER Biosoft International, CA, USA) (Table 2). PCR amplification was conducted in a 50 μl volume containing 5 μl of 10 × buffer, 1.5 mM MgCl2, 0.25 mM dNTPs, 0.2 μM each primer, 1.5 U Taq DNA polymerase (TaKaRa Biosystems) and 10 ng pooled genomic DNA which consist of DNA from ten individuals (Sham et al. 2002). The PCR conditions were as follows: an initial step at 95 °C for 5 min, 35 cycles for 35 s at 94 °C, 35 s at a specific annealing temperature for each primer pair (Table 2), and 35 s at 72 °C, followed by a final extension for 10 min at 72 °C. PCR products from the pooled DNA samples were sequenced directly by an ABI PRIZM 377 DNA sequencer (Perkin-Elmer). DNA sequences were edited using the DNASTAR 5.0 package (DNAstar, Madison, Wis., USA) and aligned by ClustalX version 2.0 (Larkin et al., 2007).

2.3. Genotyping by PCR-RFLP

After identifying all the SNPs from the horse genomic DNA pools, PCR-RFLP protocols were designed to identify and genotype SNPs in the 514 samples. Restriction enzymes including *Rsa*I, *Ssp*I, *Tas*I, *Bsm*I, *Acc*I and *Sca*I were chosen to screen the SNPs. The RFLP condition was as follows: 5 μl of PCR products was digested with 3U of restriction

Table 2
Primers, PCR conditions, amplified regions, SNP location and restriction enzyme information.

Primer pair	Primers (5′ → 3′)	Amplified region	Products length (bp)	Annealing temperature	SNP	Restriction enzymes
P1	1 F:TCAGGGAAACAAGTTTCTCAAAT	Promoter	484	63.2 °C	g.26 T > C	Rsa I
	1R: TGCTCCACAATGAATCTCG	Promoter				
P2	2 F: GACTTGTGACAGACAGGGTT	Promoter	457	56.6 °C	–	–
	2R: CGCAGTTTACTGAGGATTT	Exon 1				
P3	3 F: TGCTGATTCTTGCTGGTCC	Exon 1	325	55.6 °C	–	–
	3R: GGCATGGTAATGATTGTTTC	Exon 1				
P4	4 F: CGACGACGGAAACAATCAT	Exon 1	415	56.6 °C	–	–
	4R: TTAGGCAACCAAACGCAAT	Intron 1				
P5	5 F: CATAATTGCGTTTGGTTGC	Intron 1	467	57.8 °C	g.1485C > T	Acc I
	5R: CCTCCCTCCCAAGAAGAATA	Intron 1				
P6	6 F: AGGCAGGCACATTGCTTAAT	Intron 1	480	56.6 °C	-	-
	6R: GAATGTTATATTCAGGCTATCTCAA	Intron 1				
P7	7 F: CTAACTTTTGAGATAGCCTG	Intron 1	366	59 °C	-	-
	7R: CCAGAAAACTGTGAACTAAG	Intron 1				
P8	8 F: ATGTTCCTCCACGGTGTCT	Intron 1	446	56.6 °C	–	–
	8R: GGGCCTTTACTACTTTATTG	Exon 2				
P9	9 F: GCAAGTGGAAGGAAAACCCA	Exon 2	381	65 °C	-	-
	9R: TATTTTCATTTATCACTTACC	Exon 2				
P10	1 F: TCAGGGAAACAAGTTTCTCAAAT	Promoter	204	50.7 °C	g.156 T > C	Ssp (CRS)
	10R: ACTTCCTCAGAAATTAAGATTTAAT	Promoter				
P11	2 F: GACTTGTGACAGACAGGGTT	5′-UTR	322	60 °C	g.587 A > G	Tas I
	11R: GGACCAGCAAGAATCAGCAC	5′-UTR				
P12	2 F: GACTTGTGACAGACAGGGTT	5′-UTR	215	51.6 °C	g.598C > T	Bsm I (CRS)
	12R: TACTTTTCTTTTGCTTTTGAGGAAT	5′-UTR				
P13	6 F: AGGCAGGCACATTGCTTAAT	Intron 1	330	55.4 °C	g.2115 A > G	Sca I (CRS)
	13R: GCAGAGTCATAAAGGAAAAGTA	Intron 1				

Note: Primer pairs 1, 7 and 9 were from references (Baron et al., 2012; Dall'Olio et al., 2010); Primer pairs 2–6 and 8 were designed based on horse MSTN sequence (GenBank No. GQ183900.1). CRS: created restriction site.