DOI: https://doi.org/10.5564/mjas.v27i02.1278

Partial sequencing the exon 1 of *MSTN* in Mongolian horse *(EQUUS CABALLUS)*

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ABSTRACT

Myostatin (MSTN) is a protein responsible for muscular tissue differentiation, development, and growth in mammals. Studies on cattle, mice, pig, and dog have provided sample demonstrations of mutations on myostatin, which strongly affected animal Ankole93200phenotype, particularly muscle development. In horse, myostatin gene is usually studied with respect to racing performance. Most Mongolian native horses do race for long distance and such racing is a very popular sport in Mongolia. On the other hand, the MSTN gene also affects meat yield and quality. We partially sequenced of MSTN gene to explore its polymorphic features in Mongolian horse. A 238 bp long segment of exon 1 was sequenced, using 3130 Genetic Analyser and MEGAX. The sequence was registered with accession number LC216412 in the Gene bank database of NCBI and aligned to four reference sequences (GQ183900.1, KC708233.1, AY840554.2, NM 001081817) from this database. The nucleotide diversity was 0.0084, Tajima test statistic was -1.12. so that there were 233 invariable (monomorphic) sites and 5 variable (polymorphic) sites, in the group of animals compared. The Mongolian horse differed from the reference sequence at 3 sites. There was an amino acid difference in the partial sequence, in which isoleucine in the reference sequence was converted into valine. The results of the study indicate that the Mongolian horse seems to differ substantially from the reference genome of equus caballus. Sequencing of the full length of exon 1 or even the whole MSTN gene, including introns, is strongly advised, to get more insights with regard to the identification and characterization of variants at the myostatin locus in Mongolian horses.

KEYWORDS: Tes horse, MSTN gene, partial sequencing

INTRODUCTION

Myostatin is growth factor which regulates myogenesis by inhibiting myoblast differentiation into myotube, thus affects the muscle tissue formation and muscle cell proliferation in mammals. Myostatin The Myostatin encoded by the *MSTN* gene, and *MSTN* gene in the growth and development of Thoroughbred skeletal muscle for racing performance has been observed [1, 7]. The *MSTN* gene, composed of three exons and two introns, and most commonly studied gene with regards to horse racing performance. Several studies have been carried out to reveal the association of myostatin gene and SNPs (single nucleotide polymorphisms) on phenotype characteristics of different horse breeds. Hill [6-7] first discovered SNP at location of g.66493737C<T which was strongly associated with optimal race distance in Thoroughbred horse. Additional findings of new SNPs [4-5] and insertion element (227bp) [6] were determined in the horse myostatin gene region. Another three SNPs in MSTN gene (g.65809482T>C, g.65868604G>T, g.66539967A>G) were identified and examined on its association with horse lifetime earnings on Japanese Thoroughbred [4]. Haplotype segregation analysis of these three SNPs were studied, and T-G-C haplotype showed the most significant association with lifetime earnings. Haplotype diversity of these SNPs could be genetic marker for horse race ability [5, 7-8].

A study on 16 different horse breeds identified seven SNPs in the region of MSTN gene [9]. Suggesting two SNPs at promotor region of myostatin could affect horse morphological difference, yet it is not confirmed due to the possible relatedness of horses in same stables. Sequencing of exon 1 of *MSTN* gene in camel by Shan [10] showed the high degree of homozygosity among different species (pig-98%, cattle-94%, sheep-94%) confirming the reserved nature of MSTN gene in different species [10].

Another study of the *MSTN* gene in which the encoding amino acids of the gene revealed. The Myostatin of the full-length 375-amino acids in

human recombinant myostatin and 110-amino acid carboxy-terminal proteins were examined on "***in-vitro"*** mouse skeletal muscle C_1C_{12} cells and both myostatin gene recombinants were inhibiting C_1C_{12} cells proliferation [2].

Mongolian horse refers to middle and small-sized horses according to Japanese horse classification standard, however it belongs to pony by international classification or European standard [11]. Local horse in Mongolia classified into two breeds, Khalkh and Galshar; three lines; Tes, Myangad, and Darkhad namely; regarding their linear measurements, geographical differences, body conformation and physical appearance [12-13]. Due to its role of muscular tissue development in mammals (cattle, sheep, dog, pig, etc.) myostatin is the main candidate for animal economic traits; cattle and pig meat production. Among horse, it is a main candidate for horse physical performance. Therefore, through this study, we did a partial sequencing on 238 bp of exon 1 of *MSTN* gene in Tes horse in Mongolia. We aligned to four sequences of the *MSTN* gene from Gene bank database. The *MSTN gene of* Mongolian horses not studied previously; therefore, the study will be positive influence in other study in Mongolian horse breeds for racing, milk and meat productions.

MATERIALS AND METHODS

Blood samples from four unrelated horses in Tes sum, Uvs province were collected from four different locations.

DNA extraction

Horse genomic DNA was extracted using as a phenol-chloroform method. DNA extraction procedure as follows: 450 µl of lysis buffer (0.1 M Tris-HCl pH 8.0, 1% SDS. 0.1 M NaCl, 10mM EDTA), 5 µl proteinase K (10mg/ml) were added into 1.5 ml Eppendorf tube with 150 µl blood sample. The mixture was incubated at 55^0C for two hours. Every 30 minute the mixture was shaken softly by hand. 500 µl phenol chloroform was added and vortexed for 2 minutes and centrifuged at 15000 rpm for 5 minutes. The supernatant was collected into new tube and 40 µl NaCl was added in. 1 ml cold ethanol was added and put at -20°C for 30 minutes. Then centrifuged at 15000 rpm for 10 minutes. Upper liquid was discarded and 100 µl, 70% ethanol was added into the pellet and centrifuged at 15000 rpm for 5 minutes. Again, the ethanol is discarded, and DNA pellet is dried in the air for 10 – 20 minutes and dissolved in 20 µl TE (10 mM Tris HCl, pH 8.0, 1 mM EDTA) then stored at -20°C. DNA concentration and purification were determined using NanoDrop 1000 spectrophotometer.

PCR and Sequencing

To amplify 238 bp exon 1 of myostatin gene, forward (5'-tgtgctgattcttgttgctggtc-3') and reverse (5'-atcaatcagttcccggatg-3') primers were used in PCR. 20 µl PCR amplification reaction included: 10 µl 10xPCR buffer, 0.5 µl Taq polymerase, 1 µl of each primer, 5 µl – ddH_2O and 2.5 µl template DNA. Primers were chosen from a study by Stefania et al (2010). Thermal cycle for PCR as follows: preheating step at 95°C for 5 minutes, denaturation step at 95°C at 30 seconds, annealing step at 56°C for 30 seconds, elongation step at 72°C for 30 seconds, with 35 cycles and final extension step at 72°C for 9 minutes. PCR results were examined on 1.5% agarose gel electrophoresis (Figure 1). Sequencing was conducted using 3130 Genetic Analyzer at Molecular Biology Laboratory of National Institute of Forensic Science in Ulaanbaatar.

Statistical Analysis

Raw sequences were analyzed by CodonCode Aligner, further comparisons within and between species were performed through NCBI-Blast and polygenetic tree was created using MEGA 6.0 software.

RESULTS AND DISCUSSION

The four sequences of *MSTN* gene was generated successfully, encompassing 238 bp (Figure 1). The sequences of *MSTN* gene was registered with accession number LC216412. Furthermore, four reference sequences (GQ183900.1, KC708233.1, AY840554.2, NM 001081817) from Gene bank database were chosen for SNP and phylogenetic tree comparison. The reference sequences contain the exon 1 of *MSTN gene.*

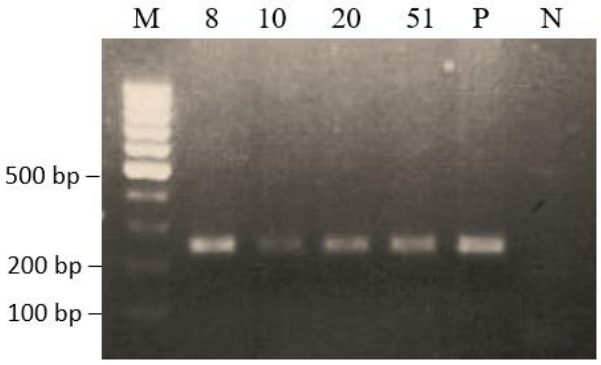

Figure 1. The PCR products of the MSTN gene in Mongolian horses
M – Marker; 8,10,20,51 – Sample number; P – positive control; N- negative control.

Table 1

A mapping of MSTN gene 238 bp of nucleotides sequences in horses the Exon 1 five segregating sites

	40	45	46	116	177
Reference sequence (GQ183900.1)	G	G	A	G	A
LC216412.1		T	G	T	
KC708233.1	A				
AY840554.2					
NM 001081817					G

The nucleotide diversity was 0.0084, Tajima test statistic was -1.124 and there are 233 invariable (monomorphic) sites and 5 variable (polymorphic) sites (Table 1, 2)

Table 2

Described result of the Exon 1 of *MSTN gene genetic diversity*

n	S	π	*D*
5	5	0.0084	-1.124

n – sample number, S – number of polymorphic sites,
π – nucleotide diversity, *D* – Tajima test statistic

Phylogenetic tree was constructed using data of same region of myostatin gene in horse breeds obtained from NCBI database.

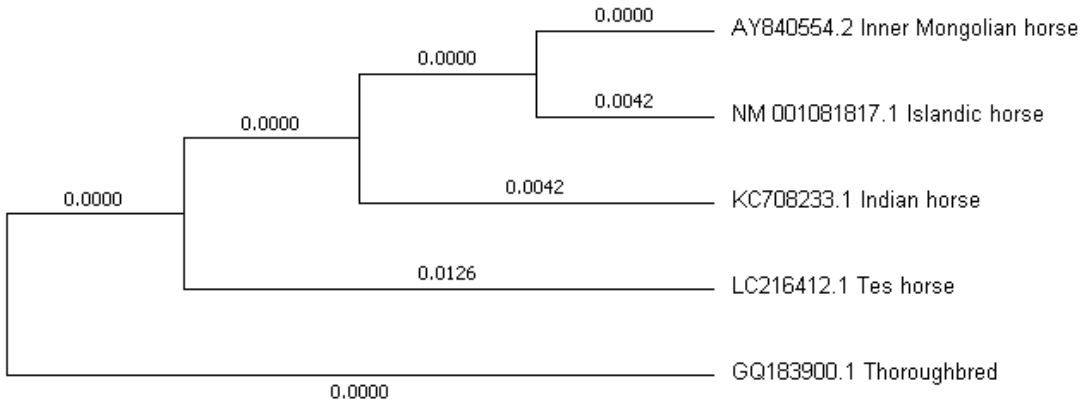

Figure 2. Phylogenetic tree constructed using
exon 1 of MSTN gene in different horse breed

In the phylogenetic tree, the genetic distance was 0.126 in Tes horse and Inner Mongolian horse, Thoroughbred, 0.168 in Tes horse and Indian horse, Islandic horse. Tes horse is closer to Inner Mongolian and Thoroughbred has the farthest relation with Indian and Islandic horse breeds which determined by genetic distance. As described by phylogenetic tree, Tes horse is closer to Inner Mongolian and Islandic horse and has the farthest relation with Indian and Thoroughbred horse breeds. Comparative closeness of genetic relationship between Mongolian and Islandic horse was determined using partial sequencing of MSTN. There are 2 amino acid difference in *MSTN gene and found* methionine into valine in Tes horse. Figure 2 shows the comparison of amino acids of exon 1 of myostatin gene in Tes horse (LC216412) and reference sequences (GQ183900.1, KC708233.1, AY840554.2, NM 001081817).

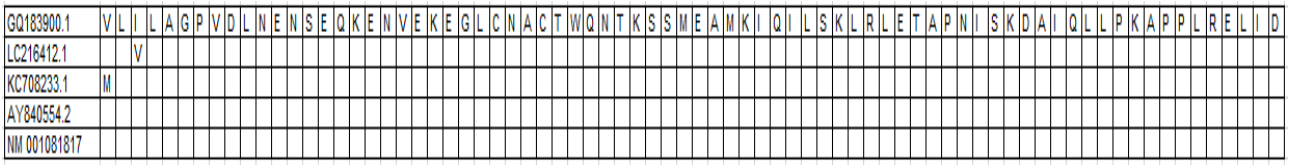

Figure 3. Amino acid comparison of exon 1 of MSTN gene between horses

CONCLUSION

1. 238 bp nucleotide sequence of exon 1 of myostatin gene in Tes horse was registered at NCBI/GenBank/Database with accession number LC216412.
2. Comparison of sequences in the same part of *MSTN* gene in Inner Mongolian horse, Through bred, Islandic horse and Indian horse revealed polymorphic sites, this led to one-amino acid differences from other breed horses. That illustrates the Exon 1 of MSTN gene in Tes horses specific from other breed horses.
3. MSTN gene of Mongolian horses different from Inner Mongolian horse for three-nucleotide sites. That result may connect the Mongolian extreme weather leads to Mongolian horse that has the ability of long racing and muscle amount, large fibre structure in meat.
4. As they suggested it is possible that Mongolian horse genetics could have penetrated to North European territory during the Mongolian invasion of central Asia. Even though our result compromises with this suggestion, so some extend, we cannot claim that it is true due to few study in horse genetics. Further analysis on genetic structure of Mongolian horse population should be genotyped to reveal the genetic differences of horse subpopulations in Mongolia. Studies to determine horse's potential genetic resource should be continued and it could be helpful and meaningful to choose individuals for particular breeding purpose such as racing ability, milk and meat yield etc.,

ACKNOWLEDGEMENT

We wish to express our sincere appreciations to Professor Johann Soelkner, who reviewed to be corrected the some part of this paper.

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