A Thesis

For the Degree of Master of Science in Veterinary Medicine

Effects of SNP in *MSTN* gene on Racing Performance of Jeju Native Horses

Department of Veterinary Medicine

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Abstracts

A sequence polymorphism (g.66493737 C>T) in *MSTN* gene, which is encoding myostatin, has been revealed that it is strongly associated with best race distance among elite Thoroughbred horses. It is assumed that *MSTN* variants in Jeju native horses are also related to racing phenotypes under the same condition. This study is designed to investigate polymorphisms present at g.66493737 locus and association between SNP and racing performances among Jeju native horses.

Blood samples were collected from 133 Jeju native horses registered in KRA. The genomic DNA was extracted from EDTA-treated whole blood. PCR primers were designed to detect *MSTN10* gene fragment (580bp) using the Primer3 primer design tool. The PCR products were cloned and sequenced to set up allele specific positive controls. Following design of the allele specific primer sets, nested PCR's were performed to identify the polymorphism present in *MSTN10*, which is a partial sequence of intron 1 region. According to the sequence variant, Jeju native horses were genotyped, and the genotype distributions were evaluated. Genotype-phenotype association analyses were also carried out: association between genotype and racing performance; association between genotype and Best Racing Distance (BRD). As a result, 5 (4.0%) out of 133 horses showed C/C genotype, 43 (32.0%) were C/T, and 85 (64.0%) were T/T, respectively. There was no significant difference between Elite Jeju native horses (JHE) and Ordinary Jeju native horses (JHO) in regard to minor allele frequency (MAF), C allele. However, C allele frequency in horses that showed BRR in short distance race (400m) was significantly higher than in horses performing well in longer distances

(>800 m).

In this study, it was possible to ascertain that a SNP (g.66493737 C>T) is present in *MSTN* gene among Jeju Native horses. In addition, it has been revealed that there is a great genetic influence in sprinting ability and suitable performance type of horses. Therefore, the established database is expected to provide a valuable reference in horse selection and future training regime for young horses.

Key words: *MSTN*, SNP, Jeju native horses, Genotype distribution, Best Racing Distance (BRD)

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I. Introduction

Myostatin is the key protein responsible for skeletal muscle growth and development (33), and is a member of the transforming growth factor β superfamily (28, 29). As a negative regulator, it acts to limit skeletal muscle mass by regulating both the number and growth of muscle fibers (28), and exerts the effects by repressing the levels of basic helix-loop-helix transcription factors MyoD, Myf5, myogenin, and MRF4, collectively known as the MyoD family muscle regulatory factors (MRFs) (24). They are known to determine the specific myogenic lineage and also critically control the differentiation of skeletal muscle cells (25, 30, 35, 37, 42).

The myostatin protein is encoded by the *MSTN* gene (previously referred to as *GDF8*) that is composed of three exons and two introns (28). The *MSTN* gene, which is highly conserved across species, has been characterized in various species such as rodents (28), humans (12), several livestock species (10, 20, 21, 40, 41), and horses (9, 17, 19, 27), and natural mutations of the *MSTN* have also been found in these species. The loci in the *MSTN*, in which these mutations take place, may be located within either coding sequences (3-5, 11, 13, 14, 22) or non-coding regulatory regions (8, 40, 45). As a consequence, the mutations exert their effects on various phenotypic traits including growth, reproduction, performance, and carcass quality. For example, in Belgian Blue and Piedmontese cattle breeds, loss-of-function mutations within the coding sequence of the *MSTN* lead to increased skeletal muscle mass, and the produced phenotype is known as “double-muscling” (13, 14, 22). Similarly, a loss-of-function mutation associated with gross muscle hypertrophy has been reported in a German baby (39). In the Whippet dog breed, a mutation in the third exon leading to a premature stop codon causes an increased muscle mass in homozygotes and enhanced racing performance in heterozygotes (32). In certain Norwegian sheep breeds, mutations in coding sequence of *MSTN* are associated with carcass conformation and fatness (4, 5). On the other hand, in other sheep breeds and pig breeds, several mutations in non-coding regulatory regions leading to altered level of *MSTN* expression (8, 40, 45).

In horse, the *MSTN*, which contains three exons and two introns spanning 6,172 bp (reverse strand nt 66489608 – 66495780, EquCab2.0) (43), has been mapped to equine chromosome 18 though development of comparative mapping technique using somatic cell hybrid analysis (6). Recently, a number of polymorphisms in the equine *MSTN* gene have been identified in Thoroughbred horses via a genome-wide screening process (17, 19), and it has been reported that a single nucleotide polymorphism, SNP, (g.66493737 C>T) in intron 1 of the equine *MSTN* gene, namely *MSTN10*, is strongly associated with optimum racing distance in Thoroughbred horses. Among horses that compete preferably in short distance races requiring exceptional speed, the C allele frequency was significantly higher than among horses that perform optimally in longer distance races that require more stamina (17). A similar pattern was shown in investigations of the genotype frequencies among non-Thoroughbred horse populations. The T/T genotype was remarkably predominant in Egyptian Arabian Horse, which is the breed known for endurance exercise, while C allele frequency was significantly higher in a breed (Quarter Horse) known for short distance race and activities (17).

Racehorse industry is a multi-billion dollar international enterprise engaged in the breeding, training, and racing (17). With the growth of racing industry, populations of Korean horse have been gradually increased, and approximately 23,000 horses including 8,000 Thoroughbred horses and 15,000 other breeds (Jeju native horses and Jeju x Thoroughbred horses) are currently raised on 1,142 premises in Korea (1). Among those, the Jeju native horses comprise about 750 herds according to Jeju Province, and this breed has been selected for multi-purposes such as riding, racing and meat production (7). As a part of breed conservation programme, the Korea Racing Authority (KRA) carries out Jeju native horse races at the Jeju race park (7), and there have been numerous studies on genetic characterization of Jeju native horses using molecular biological techniques (7, 23, 44).

To-date, no genetic markers influencing athletic performances of Jeju native horses have been reported and no data of SNPs present in *MSTN* gene are available, unfortunately. Therefore, this study was designed to detect the SNP present in *MSTN10* among the Jeju native horses registered in KRA, hence investigating genotypic distribution. In addition, possible associations between the *MSTN*

sequence variants and racing phenotypes have been evaluated, and it includes determination of the optimal racing distances on the basis of race records in different racing distances.

II. Materials and Methods

1. Animals and Sampling

1) Animals

In total, 133 Jeju native horses (JH), which are racing at the Jeju Race Park, were randomly selected for this study, and these horses are accredited through a strict breed preservation programme implemented by the Livestock Policy Division, Jeju Self-governing Province.

2) Grouping

The animals were divided into 2 groups for a statistical analysis, they include elite and ordinary groups. The horses, which belong to the elite group, recorded more than 10 races and race-winning percentage over 25%, whereas the ordinary group recorded more than 10 races and race-winning percentage under 3%. In addition, the elite group was sub-divided into best racing distance less or equal to 400m (BRD≤ 400m) and best racing distance more or equal to 800m (BRD≥800m) horse groups.

3) Sampling

Whole blood of 0.5 ml in volume was sampled from 133 JH each by jugular venipuncture, and the samples were stored in anti-coagulant (EDTA-2K) treated tubes for further genomic DNA extraction.

2. Study Design

1) DNA extraction

Genomic DNA was extracted from 300 μl of fresh whole blood with a commercial G-DEX IIb Genomic DNA Extraction Kit® (Intron Biotechnology, Korea) according to the manufacturer's instructions. The DNA samples were quantified using Nanovue® (GE Healthcare Bioscience, USA), and the final DNA concentrations were set to 100 ng/μl by diluting with Tris-EDTA buffer solution. The extracted DNA templates were stored at - 70℃ until use.

2) Primary PCR

A pair of oligonucleotides was designed to amplify the *MSTN10* fragment of *Equus caballus MSTN* gene using the PCR Suite extension to the Primer3 web-based primer design tool, and the primer pair used for primary PCR is listed in Table 1. In brief, the PCR was performed using Takara PCR Thermal Cycler DICE Gradient® (Takara, Japan) in total volume of 20 μl reaction mixtures, each containing 10x PCR buffer, 10 pmol of each primer, 1 unit of *Taq* polymerase, 250 mM of dNTP, and 100 ng of genomic DNA.

In investigating the presence of *MSTN10* fragments with the size of 580bp, the results of PCR were examined by electrophoresis on 2% agarose gel (SeaKem®, Japan) and UV transillumination of ethidium bromide stained PCR products. The flanking sequence and primary PCR reaction profiles of *MSTN10* are illustrated in Figure 1 and Table 2, respectively.

Table 1. Lists of PCR primer pairs for amplification of *MSTN10* fragments of equine *MSTN* gene

Primer	Sequences (5'→ 3')	Target gene	Product Size (bp)	References
For Primary PCR				
MSTN10F0	CTTGG TGCAT TATAA CCTGA	*MSTN10*	580	[19]
MSTN10R0	GTCTGCGATCCTGCTTTACC			
For Nested PCR				
MSTN10F1	CAGAGTCATAAAGGAAAATTAT	3' Fragments of *MSTN10*	125	Present Study
MSTN10F2	CAGAGTCATAAAGGAAAATTAC			
MSTN10R1	ATCAGGTTATAATGCACCAAA	5' Fragments of *MSTN10*	497	
MSTN10R2	ATCAGGTTATAATGCACCAAG			

66493260 **TGAAGGAATGAACTGTGGATG**AAATTTTAAAATGATGATGATTAGAGAGAACAAGAGACACCGTGGAGGAACATCCACTTAGAATTCTTT

66493350 GGGAATCTGAGTAGTTACACTTACTGAGCAGCTGTACCAATCAGTCTGGAAGAAGGAACCCTTCCCCCAGGCCTGAATTACCTGGGGACA

66493440 AGACACACTGAGGAACTAACTGAGCCTCGGGAATTAAGAGAAAATATAGTACATCTGTTATGTTTTGGCTTTGGAATAGCCTTTTAAAGG

66493530 AACAAAGCTAAGCAAGTAATTAGCACAAAAATTTGAATGTTATATTCAGGCTATCTCAAAAGTTAGAAAATACTGTCTTTAGAGCCAGGC

66493620 TGTCATTGTGAGCAAAATCACTAGCAATTTCTTTTATTTTGGTTCCCCAAGATTGTTTATAAATAAGGTAAATCTACTCCAGGACTATTT

66493710 GATAGCAGAGTCATAAAGGAAAATTA**T/C**TTGGTGCATTATAACCTGATTACTTAATAAGGAGAACAATATTTTGAAACTGTTGTGTCCTGT

66493800 TTAAAGTAGATAAAGCACTG**GGTAAAGCAGGATCGCAGAC**

Figure 1. Flanking sequence of *MSTN 10* with SNP g.66493737 C>T (chr18:66493261+66493840: 580bp)

Primer pair : MSTN10F0 - R0			
	95℃	Initial Denaturation	7 mins
40 Cycles	95℃	Denaturation	30 secs
	63℃	Annealing	30 secs
	72℃	Extension	30 secs
	72℃	Final Extension	7 mins

Table 2. Lists of primary PCR reaction profiles for amplification of *MSTN10* with the size of 580bp

3) Cloning and Sequencing

To set up the positive controls corresponding to C and T allele at locus g.66493737, 5 randomly selected products from the primary PCR were cloned and sequenced. Following the gel-purification utilizing MEGA-bead agarose Gel Extraction Kit (Intron Biotechnology, Korea), the amplified PCR products from the primary PCR were ligated into a commercially available pCR2.1-TOPO vector (Invitrogen,USA) containing M13 promotor, of which the structure is shown in Figure 2. Subsequently, the ligated vector was transformed into a competent cell, namely *Escherichia coli* DH5α, and the transformed cells were cultured in LB broth, which is supplemented with 100ul/ml ampicillin, for 16 hours in advance to restoration of the plasmid using QIAGEN Plasmid Mini Kit (QIAGEN, Germany). Bidirectional DNA sequencing of PCR products was outsourced to Macrogen Inc. (Daejeon, South Korea) and carried out using AB 3730xl DNA analyzer (Applied Biosystems, Foster City, CA). The sequences of the cloned PCR products were analyzed using the BLAST program of National Center for Biotechnology Information (http://www.ncbi.nlm.gov/BLAST/) for comparison of homology to the *Equus caballus MSTN* sequence registered in GenBank (Accession Numbers AY840554.2 and GQ183900.1). According to the SNP present at locus g.66493737, the cloned PCR products were divided into C and T allele positive controls, and 500μl of LB broth containing transformed *Escherichia coli* DH5α cells of each positive controls were stored at -70℃ following addition of 500μl glycerol for further nested PCR's.

4) Nested PCR

According to the results obtained from the DNA sequence analysis, 2 different sets of C and T allele specific primer pairs were designed to detect the single base pair difference in *MSTN10* utilizing the identical primer design tool mentioned above. Consequently, 2 different sets of primer pairs were obtained: 1 set for 5' end fragment including the SNP (MSTN10F1 and MSTN10F2) and the other for 3' end fragment including the SNP of the *MSTN10* (MSTN10R1 and MSTNR2). The primer pairs

used for nested PCR's are listed in Table 1.

Using the product of primary PCR as a template, the nested PCR was performed for genotyping of each individual in comparison with the positive controls established as above. Similar to the primary PCR, the total volume of 20 μl reaction mixtures were used, and constitutions of the mixture were identical to those used in the primary PCR except the specific primer pairs. The final product sizes of the nested PCR were 497 and 125 bp for 5' and 3' end fragments, respectively. The nested PCR's were carried out using the identical equipment as in the primary PCR, and the reaction profiles for the first and second nested PCR are illustrated in Table 3 and 4, respectively.

5) Genotyping

On the basis of nested PCR results, 133 horses were divided into 3 different categories: C/C, C/T, and T/T genotypes. In this process, 3 different nested PCR were carried out for confirmation of the genotyping results. Initially, MSTN10F1/F2 primers were paired with MSTN10R0 primer so that 5' end 497 bp fragments could be amplified using the products of primary PCR as a template. This procedure was repeated using a different primer set, namely MSTN10F0 and MSTN10R1/R2 primers, to amplify 3' end 125 bp fragments of *MSTN10*. Finally, this procedure was duplicated using the genomic DNA as a template for confirmation of the results.

6) Statistical Analysis

All statistical evaluations for quality association analysis were performed using a statistics package, **SPSS v12.0.** This process included computation of sample allele frequency and calculation of deviation from *Hardy-Weinberg* equilibrium. Cohort-based trait association tests (Elite Jeju Horses vs Ordinary Jeju Horses and BRD≤400m vs BRD≥800m) were performed for the g.66493737C>T SNP using χ^2 tests with one degree of freedom, and also odds ratios and 95% CIs were calculated for these analyses.

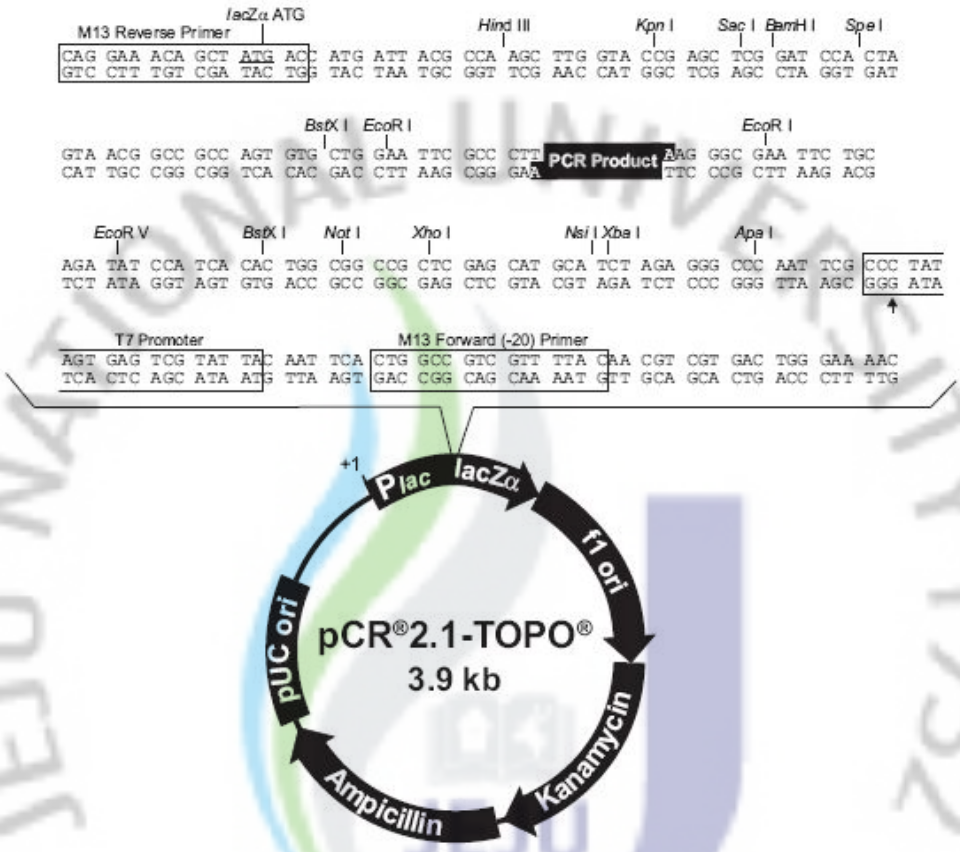

Figure 2. Genetic map of pCR2.1_TOPO vector® (Invitrogen, USA) with total vector size of 3.9kb.

Table 3. Lists of nested PCR reaction profiles for amplification of SNP 5' fragment (497bp) of *MSTN10*

Primer pair : MSTN10F0 - R1/R2			
	95℃	Initial Denaturation	7 mins
38 Cycles	95℃	Denaturation	20 secs
	65℃	Annealing	20 secs
	72℃	Extension	10 secs
	72℃	Final Extension	7 mins

Primer pair : MSTN10F1/F2 – R0			
	95℃	Initial Denaturation	7 mins
38 Cycles	95℃	Denaturation	20 secs
	62.9℃	Annealing	20 secs
	72℃	Extension	10 secs
	72℃	Final Extension	7 mins

Table 4. Lists of nested PCR reaction profiles for amplification of SNP 3' fragment (125bp) of *MSTN10*

III. Results

1. Identification of SNP g.66493737 C>T within intron 1 region of Jeju horse *MSTN* gene

MSTN10 with the total length of 580bp was amplified utilizing genomic DNA extracted from 133 Jeju horses through the primary PCR, and the results of 10 randomly selected DNA samples among 133 animals are illustrated in Figure 3. To identify the SNP at locus g.66493737, 5 randomly selected primary PCR products were cloned and sequenced. In comparison of the cloned PCR products with the *Equus caballus MSTN* sequence registered in GenBank (Accession Numbers AY840554.2 and GQ183900.1), 100 % homology was detected between them. As a result, it was possible to confirm that the PCR products are the amplified DNA fragment of target gene and they can be used as allele-specific positive controls with a single base difference present.

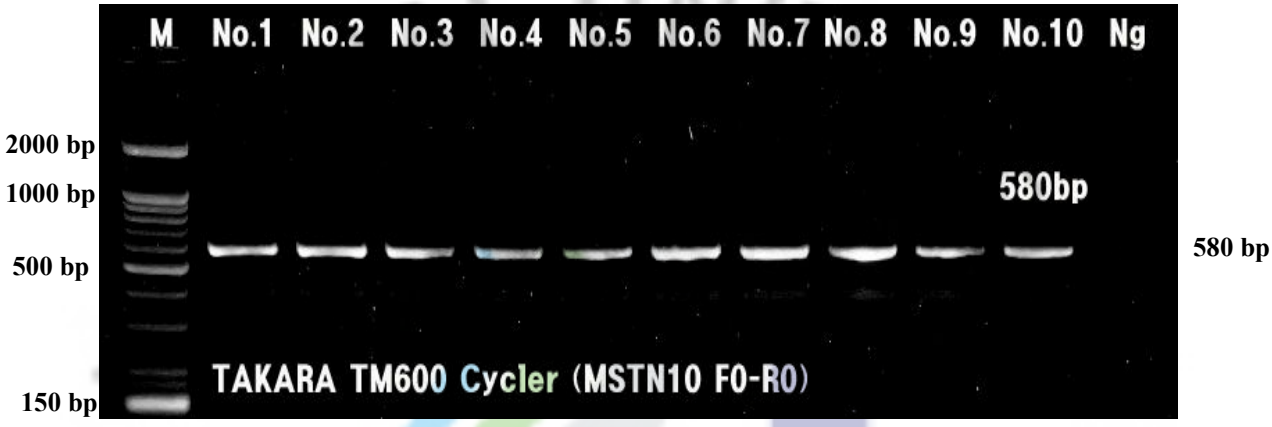

Figure 3. Primary PCR results of *MSTN10* gene in 10 Jeju native horses genomic DNA with MSTN10F0 –R0 primer set on 2% agarose gel stained with EtBr under UV light. Lane M, 25/100 bp ladder marker; Lane No.1 – No.10, amplicons of primary PCR (*MSTN10* fragments) with the size of 580 bp; Lane Ng, negative control.

2. Genotyping according to the SNP g.66493737C>T via nested PCR

To genotype the individual horses, the first and second nested PCR were performed, and the results including the C and T allele positive controls are shown in Figure 4 and 5, respectively. As a consequence, the genotypes of 133 Jeju native horses registered in KRA were identified in regard to the SNP present at locus g.66493737. Interestingly, the frequency of T/T genotype (0.64) was remarkably higher than those of other genotypes, namely C/C (0.04) and C/T (0.32) genotypes. In addition, the C allele frequency was noticeably low (0.18), and this result is rather similar to that obtained in experiments with Egyptian Arabian horses showing magnificent stamina.

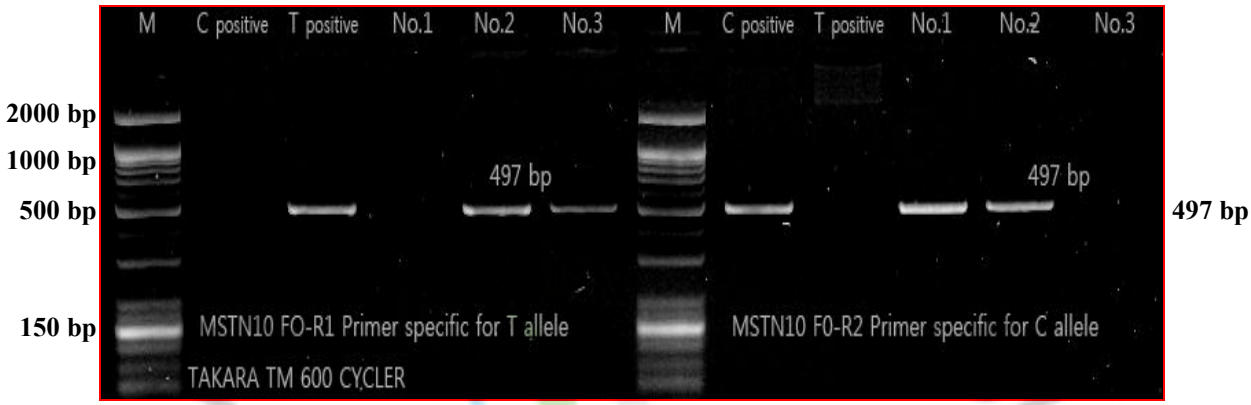

Figure 4. Nested PCR results of 5' end fragments of *MSTN10* with MSTN10F0 – R1/R2 primer sets on 2% agarose gel stained with EtBr under UV light. Lane M, 25/100 bp ladder marker; Lane $C_{positive}$, nested PCR amplicons of C allele-specific positive control; Lane $T_{positive}$, nested PCR amplicons of T allele-specific positive control; Lane No.1-No.3, genomic DNA of randomly selected Jeju native horses.

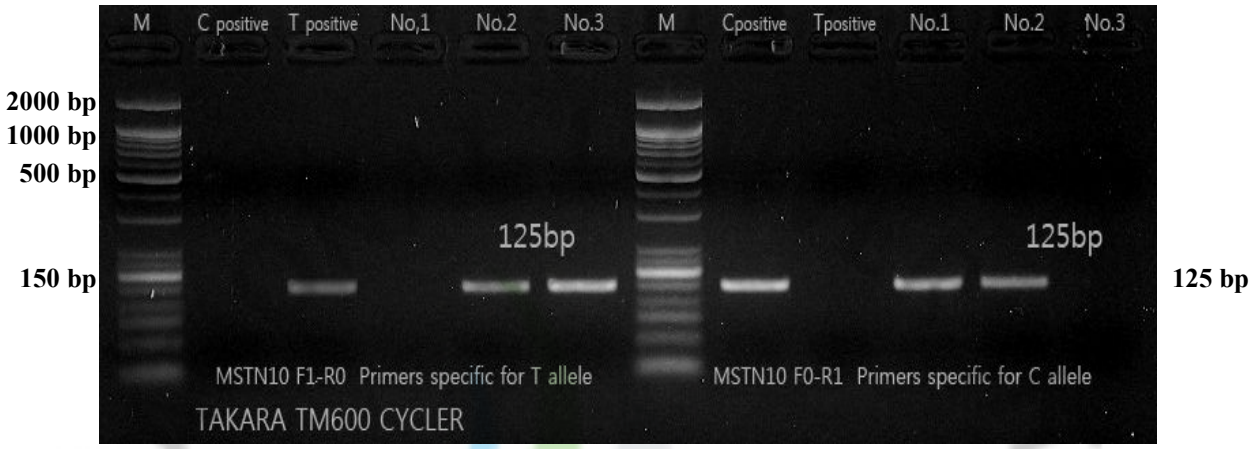

Figure 5. Nested PCR results of 3' end fragments of *MSTN10* with MSTN10F1/F2 – R0primer sets on 2% agarose gel stained with EtBr under UV light. Lane M, 25/100 bp ladder marker; Lane $C_{positive}$, nested PCR amplicons of C allele-specific positive control; Lane $T_{positive}$, nested PCR amplicons of T allele-specific positive control; Lane No.1-No.3, genomic DNA of randomly selected Jeju native horses.

3. Elite Jeju Native Horses (JHE) vs Ordinary Jeju Native Horses (JHO)

Among 133 samples, the horses, which raced more than 10 times, are divided into 2 separate cohorts according to the racing results: Elite (≥25% wins) and Ordinary (≤3% wins) horses, and a cohort-based genotype-phenotype association analysis was performed. In comparison between 35 JHO and 45 JHE, no significant differences in genotype distribution were detected. In addition, Pearson's χ^2 test (df = 1) showed that there are no significant minor allele frequency (MAF) differences between two cohorts within 95% CI, and odds ratio was also relatively low (Table 5).

Table 5. Comparisons for g.66493737C>T between Ordinary Jeju native racehorse group (JHO) and Elite Jeju native racehorse group (JHE)

	No. of animals	Genotype (C/C, C/T, T/T)	Trend (C,T)	MAF (C allele frequency)	*p*-value	OR
JHO	35	(3,13,19)	(19,51)	0.271	0.581	1.224
JHE	45	(4,13,28)	(21,69)	0.233		

JHO, Jeju native horses that recorded more than 10 races and winning percentage under 3%; JHE, Jeju native horses that recorded more than 10 races and winning percentage over 25%; MAF, minor allele frequency; *p*-value obtained from Pearson's $\chi 2$ test (degree of freedom = 1); OR, odds ratio calculated for quantitative association analysis.

4. JHE Best Racing Distance (BRD) ≤400m vs JHE Best Racing Distance (BRD)≥800m

Considering best race distance (BRD) as a phenotypic variable, the 45 elite group horses were subdivided into 2 groups: 16 short (BRD ≤ 400m) and 29 middle-long (BRD ≥ 800m) distance race winners. The C allele frequencies, minor allele frequency (MAF), of short distance (BRD ≤ 400m) and long distance (BRD ≥ 800m) cohort were 0.281 and 0.086, respectively. In genotype-phenotype association analysis using Pearson's χ^2 test (df = 1) and odds ratio calculation, *p*-value was less than 0.05 ($p = 0.014$) and the corresponding odds ratio was 4.148. It tells that C allele is significantly more frequent in horses performing better in short distance races. In addition, C/C genotype was absent in middle-long race winner cohorts, which reinforces the results above (Table 6).

Table 6. Comparisons for g.66493737C>T between Elite Jeju native horses with their best performance in shorter distances and Elite Jeju native horse with their best performance in longer distances

	No. of animals	Genotype (C/C, C/T, T/T)	Trend (C,T)	MAF (C allele frequency)	*p*-value	OR
BRD≤400m	16	(2,5,9)	(9,23)	0.281	0.014	4.148
BRD≥800m	29	(0,5,24)	(5,53)	0.086		

BRD≤400m, Jeju native horses that recorded their best performance in races shorter or equal to 400m; BRD≥800m, Jeju native horses that recorded their best performance in races longer or equal to 800m; MAF, minor allele frequency; *p*-value obtained from Pearson's $\chi 2$ test (df=1); OR, odds ratio calculated for quantitative association analysis.

IV. Discussion

Myostatin negatively regulates the number and development of skeletal muscle fibers, and various natural sequence variants causing phenotypic changes have been reported in a wide range of animal species so far. Similarly, numerous studies of genetic influences on racing performances are undergoing in horses at present, and SNP g.66493737 C>T was only targeted as the strongest candidate in deciding the best race distances for Jeju native horses in this paper.

Following identification of the SNP in Jeju native horses, the horses were genotyped and categorized according to the SNP. In frequency calculation, T/T genotype was the most frequent genotype (0.64) followed by C/T genotype (0.32). On the other hand, the frequency of C/C genotype was extremely low (0.05) showing a similar pattern observed in Egyptian Arabian horse breed suited for endurance events. Additionally, the investigation of allele frequencies revealed that the C allele frequency (0.18) is much lower than T allele (0.82) in n = 133 Jeju horses. It may indicate that there have been natural selective forces in Jeju horse *MSTN* favoring stamina rather than speed, as observed in the recent evolution of human athletes (2) (38) (34).

In comparison between JHE and JHO groups, individual genotypes at the SNP used for the analysis were not more common among elite group horses than other ordinary horses, and also the results were not affected by applying corrective factors, such as sex and age, into the equations. It may implicate that the genuine racing ability is not affected by the SNP at locus g.66493737.

Considering the relative contribution of muscle power to sprint and longer distance racing, JHE BRD≤400m and JHE BRD≥800m groups were compared. The identical quantitative association analysis was performed in regard to allele frequency, and a highly significant association between genotype and BRD was present. C allele was more frequent in JHE BRD≤400m, whereas T allele was more frequent in JHE BRD≥800m group. This trend was more clearly shown in the calculation of odds ratio (4.148), meaning that there is a significant association between BRD's and specific allelic presentations. These results correlate well with those obtained in previous studies carried out using

Thoroughbred horses (17), and it indicates that the racing distances over 800m may be considered as longer distances requiring a high level of stamina in Jeju native horses.

Comparing to previous studies in which the genotypic variations affecting a wide range of phenotypic changes are revealed on the basis of DNA sequencing and quantitative association analysis among equine populations(9, 17-19, 26, 27, 36), this study was focused to evaluate only the targeted SNP that is known to be the most significant in determination of the best race distances via specific primer design and subsequent nested PCR. DNA sequencing may provide an opportunity to screen numerous candidate variants in an interested gene at one swoop, this process can be relatively labor-intensive and time-consuming. However, nested PCR method would rather provide a quicker and cost-effective genotyping tool as long as the targeted gene and sequence variants are accurately identified. This method can also be characterized as relatively simple and almost non-invasive, while providing a reliable result promptly.

As revealed in previous studies, the high degree of sequence conservation in animals ranging from mammals to birds to fish suggests that the biological function of myostatin is well conserved throughout the animal kingdom, indeed (19). Myostatin is a negative regulator of skeletal muscle growth and development as mentioned above, and this is mediated through down-regulation of MyoD family muscle regulatory factors (MRFs), which are known as helix-loop-helix transcription factors such as *MyoD*, *Myf5*, myogenin, and MRF4 (24). Therefore, any sequence variations causing alterations in myostatin expression lead to striking phenotypic changes in those vertebrates. However, double muscling phenotypes found in a number of different species have not been observed in horses, although it has been advocated that C/C genotype individuals display a marginally greater mass-to-height ratio than T/T genotypes (17). This may reflect that sprint racing horses are generally more compact requiring more muscle power than horses suited to longer distance races.

Alex Hennebry *et al*. have described that myostatin is involved in determination of skeletal muscle fiber-type composition by regulating myocyte enhancer factor 2 (MEF2) and *MyoD* gene expression (16). Both positively-regulated MEF2 and Negatively-regulated *MyoD* increase the level of type IIB fibres while decreasing type IIA and type I. The decrease in slow and fast oxidative fibers with a

concomitant increase in fast glycolytic fibers consequently increases the susceptibility to muscle fatigue, and these developmental changes may be determined during the fetal stages due to an alteration in myoblast specification (31). This hypothesis may address the questions for mechanisms by which the g.66493737C>T sequence variant may affect the muscle phenotype in horses, as the SNP is located within the sequence of a putative E2F family of transcription binding site in intron 1 of the *MSTN* gene. Therefore, allele-specific binding of E2F to myostatin may influence the growth and development of myocytes, regulating the number as well as the type of specific muscle fibers (15). In summary, it is possible to propose that C/C genotype horses are more compact and possess a higher degree of type IIB glycolytic fiber types, hence are outstanding in speed but susceptible to muscle fatigue, vice versa.

Further studies on genotype-specific gene expression and clinical analyses including histological examination of specific muscle groups in different genotype animals will shed light on the allele-specific effect on function. Moreover, repeated experiments with larger number of samples would increase the power of study, while prospective studies involving a series of case–control and quantitative association tests would reveal the pure contribution of genetic components on athletic phenotypes of Jeju native horses.

V.Conclusion

This study represents the first investigation of sequence variant in association with athletic performance phenotype in Jeju native horses. In this study, a rapid and reliable genotyping of KRA-registered Jeju native horses was undertaken according to the SNP g.66493737C>T via nested PCR, and it has been confirmed that C allele is more frequently found in horses suited for sprinting while T allele is more frequently found in horses suited for longer distances. It is in an agreement with the results from Thoroughbred horses, and the genetic information obtained may provide a valuable reference for determination of optimal racing distance, hence designing further training scheme for a particular individual.

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국문초록

MSTN 유전자 내의 단일염기다형성이 제주 재래마의 경주 능력에 미치는 영향

송정환

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MSTN 유전자는 근섬유의 수와 성장을 조절하는 myostatin 단백질을 합성하며, 이 유전자 내에 존재하는 다형성은 성장, 번식능력, 육질 등 다양한 형질들에 영향을 미친다. 말에서 이 유전자 내의 단일염기다형성(g.66493737 C>T)이 우수 더러브렛 경주마에서 최고 성적 거리와 관련이 있다. 본 연구에서는 제주 재래마에서 *MSTN*의 유전적 다형성이 경주 능력과 관련이 있을 것이라는 가설을 세우고, 경마에 이용되는 제주마에서 *MSTN*의 유전적 다형성과 경주 능력 간의 상관 관계를 조사하였다.

제주 경마공원 내에서 유사한 조건의 영양 공급과 훈련양을 가지는 제주 재래마 133마리를 대상으로 하였다. 경정맥을 통해서 채혈하여 genomic DNA를 추출하였다. *MSTN* 특이 프라이머 디자인과 Nested-PCR을 통해 유전형을 분류하였고, 유전형 분포를 조사하였다. 경주 능력과 유전형과의 상관관계를 알아보기 위해 기존의 경주 성적을 토대로 통계학적 분석을 실시하였다.

총 133개의 시료 중, 5(4%)마리가 C/C형, 43(32%)마리가 C/T형, 85(64%)마리가 T/T형으로 조사되었다. *MSTN* 유전형과 경주능력 간의 상관관계에서 우수마와 일반마 간의 유의적인 차이는 존재하지 않았다. 그러나, 유전형과 적합한 경주 거리와의 상관관계에서 우수마 중 단거리(400 미터 이하) 성적 우수마 군에서는 C allele가, 장거리 (800 미터 이상) 성적 우수마 군에서는 T allele가 유의적으로 높게 나타났다.

이상의 결과로, 제주 재래마에서 *MSTN* 유전자 내 g.66493737 위치에 단일염기다형성이 존재함을 확인하였다. *MSTN* 단일염기다형성이 제주 재래마에서

다양한 후천적 경주능력 결정 인자들 이외에서 적합한 경주 거리를 결정하는데 중요한 지표로 활용될 수 있음을 알았다.

주요어: *MSTN*, 단일염기다형성, 제주마, 유전형 분포, 적정 경주 거리

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