Veterinary Medicine and Science

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Equine

The Effect of Selection on the Two Important Myostatin Gene Mutations in the Dareshouri Horse in the Middle East

Reihane Seifi Moroudi[1] | Hossein Mahboudi[2] | Fereidoun Mahboudi[1]

[1]Taraz Behstour Daylaman horse stud farm, Karaj, Iran | [2]Department of Biotechnology, School of Pharmacy, Alborz University of Medical Sciences, Karaj, Iran

Correspondence: Reihane Seifi Moroudi (Reihane.seifi@gmail.com)

Received: 21 May 2024 | **Revised:** 12 February 2025 | **Accepted:** 7 March 2025

Funding: The authors received no specific funding for this work.

Keywords: breed | Dareshouri | horse | MSTN | myostatin | SINE insertion | SNP

ABSTRACT

Objective: The Dareshouri horse breed is one of Iran's native equine breeds, originating from the Dareshouri tribe, a subgroup of the Qashqai nomads. This breed has a history spanning over 500 years. Horses of this breed have smooth nates, tall stature, raised tails and strong skeletal muscles. This is the first study to investigate the effect of genetics on athletic performance in the Dareshouri breed.

Methods: For this purpose, in this study, the genotype combination of two important variants, including the rs397152648Single nucleotide polymorphism (SNP) and Short interspersed nuclear element (SINE) insertion, was surveyed to study the effect of selection on *MSTN* gene mutations in this breed.

Results: The result displayed absence of SINE insertion and high frequency of the "C" allele in *Myostatin* gene (*MSTN)* gene in all studied horses. Considering the importance of the presence and high frequency of the rs397152648 SNP and its relationship with the low expression of equine *Myostatin*, the muscle mass ratio, and speed performance, this evidence confirms that the Dareshouri breed has the genetic potential to cover the race distance in a shorter time.

Conclusion: Given the absence of genetics studies on this valuable Iranian breed, these findings represent an important contribution to the characterisation of this breed and clearly indicate an occasion for Dareshouri horsebreeders, owners, and trainers to recognise the genetic potential of their horse to make the best decisions in breeding, selection, training and racing to improve the horse's performance.

1 | Introduction

Dareshouri breed is a small population of Iranian native horses that, due to the absence of a study in the background of this breed, has not been proven to be the origin of this breed. There are only some documents that require genetic studies to prove them. The age of this breed dates back more than 500 years since the Qashqai nomads used them for long-distance migration in the mountains. The Dareshouri tribe is one of the subgroups of Qashqai nomads. Consequently, the native horse used by this tribe was called the Dareshouri horse. The horses of this breed have a smooth natis, raised tails and strong skeletal muscle and are taller than the Arabian horse (Figure 1).

Although, thus far, Dareshouri horses have been known as endurance horses due to their ability to cover long annual distances (~800 km) in the mountains, and the horses training has been done in order to participate in festivals and national

I confirm that had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

FIGURE 1 | Dareshouri horse breed.

endurance competitions. Since no genetic studies have been performed for this purpose on the Dareshouri horse breed, it is necessary to demonstrate this claim.

Up to now, numerous research studies conducted on competing horses have determined that the athletic phenotype is highly influenced by genetics, training and environment.

Several genetic factors are related to athletic ability in horses, and the *Myostatin* gene (*MSTN*) was the first gene to be investigated. Recent studies have focused extensively on the effect of this gene on horse racing performance.

Myostatin gene, a member of the transforming growth factor-beta superfamily, codifies the Growth Differentiation Factor 8 (GDF8), which is a negative regulator of muscle growth (Binns et al. 2010). The *MSTN* regulatory network decreases the proliferation rate of equine muscle precursor cells by inhibiting cell cycle progression (Thomas et al. 2000). Furthermore, it is involved in signalling pathways (Notch, Mitogen-Activated Protein Kinase signaling pathway and wingless-type MMTV integration site family (Wnt) signaling pathway), cell differentiation, glucose metabolism and adipocyte proliferation, regulation and homeostasis of skeletal muscle and cardiomyocyte, formation and renewal of bone and skeletal muscle (Grobet et al. 1997, Kellum et al. 2009, Hamrick et al. 2010, Elkasrawy and Hamrick 2010, Elliott et al. 2012, Budsuren et al. 2022).

Homologous sequences of the *MSTN* gene have been found in mammals, and research has shown that this gene is extremely conserved among many species (McPherron and Lee 1997).

Although the *MSTN* gene inhibits the proliferation rate of muscle cells, much research on animals such as mice, cattle, sheep, dogs and humans demonstrated that mutation in *MSTN* causes muscle development (Grobet et al. 1997, McPherron and Lee 1997, Schuelke et al. 2004, Mosher et al. 2005, Stinckens et al. 2010). In dogs and cattle, the increase in skeletal muscle mass due to mutation in this gene is very obvious (McPherron and Lee 1997, Mosher et al. 2005).

Until now, one SINE insertion in the promoter region (upstream of exon 1) and more than 23 SNPs have been identified in the equine *MSTN* gene (two in the promoter, eight in intron 1, one in intron 2, 10 in exon 2 and two in the 3′-UTR) (Dall'Olio et al. 2010, Hill et al. 2010).

Research on the equine *MSTN* gene has shown that two of these mutations (SINE insertion and rs397152648 SNP) affect muscle fibre ratio and are associated with racing phenotypes (Petersen et al. 2013).

Some Genome wide association studies (GWAS) on the Illumina Equine SNP50 and SNP70 genotyping array have identified the rs397152648 SNP (previously identified as g.66493737 T/C or g.2115A>G) in intron 1, which is associated with the best race distance (BRD) in Thoroughbreds, and it is a powerful athletic performance predictor. In this research, it has been reported that rs397152648 C/C horses perform better in short-distance races, T/C in middle-distance races, and T/T horses in long-distance races (Binns et al. 2010, Hill et al. 2010, Tozaki et al. 2010).

In addition, in some studies, the expression of the *Myostatin* gene in different genotypes has been examined; it has been proven that

MSTN genotypes in rs397152648 SNP affect *MSTN* gene expression. Such that horses with the *C/C* genotype had the lowest level of *MSTN* gene expression, horses with the *T/T* genotype had the highest level, and horses with the *C/T* genotype had an average level of expression of this gene (McGivney et al. 2012).

The other important Thoroughbred *MSTN* variant is a non-coding transposable element (a 227 bp SINE insertion) within the upstream of *MSTN* exon 1, in the promoter region, which was related to the distance of the race (Rooney et al. 2018). Studies illustrated that SINE insertion, such as the rs397152648 SNP, is related to the expression level of the *MSTN* gene and the muscle fibre ratio in Thoroughbred horses (Santagostino et al. 2015). The Sine insertion and rs397152648 SNP and their relationship with the racing performance in the Quarter horses also had similar results. SINE allele frequencies in Quarter horses and Thoroughbreds were reported as 0.51 and 0.81, respectively (Petersen et al. 2014, Peters et al. 2020).

In a study in Quarter horses and Thoroughbreds, it has been confirmed that horses with the *C/C* genotype or the SINE insertion have a higher ratio of type IIX (IIB) muscle fibres, and horses with the *T/T* genotype and horses without the SINE insertion have a higher ratio of type I muscle fibres and type IIA muscle fibres. Since type I fibres are oxidative, slow-twitch fibres with high mitochondrial and capillary density, type IIA fibres are fast-twitch, intermediate in their oxidative and glycolytic capacities, and type IIX fibres are glycolytic, fast-twitch fibres with low mitochondrial and capillary density, It is quite clear that horses with the *C/C* genotype or two SINE insertion alleles that have best performance in short-distance races require fast-twitch muscle fibres and horses with T/T genotype and without two SINE insertion alleles that perform better in long-distance races require higher oxidative capacity and mitochondrial density and slow-twitch muscle fibres (Petersen et al. 2013, Petersen et al. 2014, Tozaki et al. 2011, Rooney et al. 2017).

Since it is well established in Thoroughbred, Quarter Horse, and some of the other horse breeds that these two *MSTN* variants (rs397152648 SNP and SINE insertion) are of particular importance and affect the race performance. Because no study has been performed on these important *MSTN* variants in the Dareshouri horse breed, the purpose of this study was to survey the genotype combination of these two important variants in Dareshouri horses to pursue the effect of selection on *MSTN* gene mutations in this breed.

2 | Materials and Methods

2.1 | Study Animals

In this study, hair samples of 52 Dareshouri horses were collected from 3 different farms: Chapar stud farm located in Semirom, Isfahan, Iran; Taraz Behstour Daylaman stud farm, located in Gilan province in Iran and Salehieh farm, located in Alborz province in Iran. All the samples were gathered based on the pedigree information from the herdsmen, and to avoid the individual's connection, additional horse samples (n = 45) from different farm, were added to this study.

TABLE 1 | Specific primer sequence for MSTN promoter sequence.

Primer	Sequence (5'-3')
MSTN-F	ATCAGCTCACCCTTGACTGTAAC
MSTN-R	TCATCTCTCTGGACATCGTACTG

2.2 | DNA Extraction and Genotyping

Genomic DNA was isolated from hair roots following standard procedures of the GenUP gDNA Kit (Biotechrabbit, Germany). In order to extract high-quality DNA and prevent mixing of samples, special criteria were considered, and the protocol was modified somewhat. The original protocol stated to wash and centrifuge the DNA twice. We performed an additional washing step, and in the final step, we centrifuged at 13,000 rpm for 1 min instead of 10,000 rpm for 1 min.

For genotype data collection, all hair samples were genotyped using Illumina SNP70 BeadChips.

2.3 | Statistical Analyses

To check the individual's relationship, the selection was made based on the registered pedigree information in each farm, and then genotyping data were used.

At first, PED and MAP files were industrialised using R software (v R-4.3.2). Then, quality control (QC) was performed using PLINK (v1.07) software. MAF over 0.01 (1%) and genotyping rate > 95% were considered. Then. to find the individuals with a similar genetic base, PI-HAT values were calculated in PLINK to estimate a coefficient of IBS/IBD for each individual. Afterwards, the genotype data were surveyed for *MSTN* SNP by coding in R software.

2.4 | Polymerase Chain Reaction (PCR) Amplification and MSTN Genotyping

To investigate the presence of the 227 base pair SINE insertion in the *MSTN* promoter, the SINE insertion was genotyped using polymerase chain reaction (PCR) based on a pair of specific primers (Table 1) reported in a previous study (Hill et al. 2010). The primers were purchased in lyophilised form and according to the instructions of each primer after diluting, were stored at −20°C.

The PCR reaction was performed in a 20 μL volume including 3 μL of DNA sample (50-150 ng /uL), 1μL of each primer (1 pM) and 15 μL Master Mix Ampliqon 2X.

The PCR conditions were as follows: an initial step at 95°C for 5 min in the first denaturation step, 35 cycles of 30 s at 95°C, 30 s at 58°C, and 1 min at 72°C for the denaturation, annealing and extension steps, respectively, followed by a final extension for 9 min at 72°C.

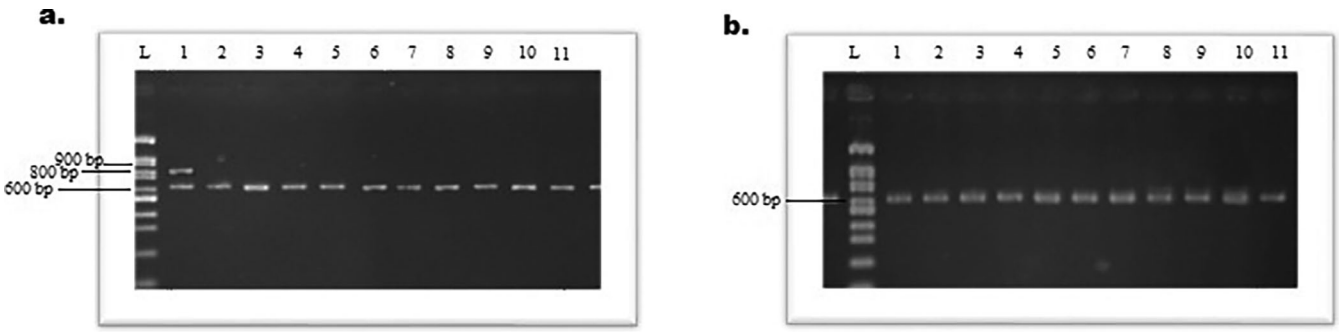

FIGURE 2 | Polymerase chain reaction (PCR) amplification of the target fragments of the equine *MSTN* genes (SINE insertion) on 2% agarose gel: **(a)** PCR products of related Dareshouri horses (wild type or *N/N* genotype) and Thoroughbred horse (heterozygous or *I/N* genotype). L, DNA Ladder; 1, Control (PCR product of Thoroughbred), 2–11, PCR products of related Dareshouri horses. and **(b)** PCR products of unrelated Dareshouri horses (wild type or *N/N* genotype). L, DNA Ladder; 1–11, PCR products of unrelated Dareshouri horses.

Amplicons were checked for amplification on 2% agarose gel. Genotyping was completed based on the determination of the size of amplicons on agarose gel. Two 600 bp fragments indicate to horses without SINE mutation (wild type or *N/N* genotype), one 600 bp fragment and one 827 bp fragment refer to horses with one SINE insertion (heterozygous or *I/N* genotype), and two 827 bp fragments relate to horses with two SINE insertions (homozygous or *I/I* genotype).

3 | Results

All Dareshouri horses were genotyped with the Illumina Equine SNP70 BeadChips. To avoid pedigree-based bias, two groups of samples were genotyped. Group 1 included related horses (within two generations) (n = 34), and group 2 was (n = 63) unrelated horses from a larger DNA sample; the horses of each group were selected based on pedigree information and genotype analysis. For all genotype data after quality control, the PI-HAT coefficient was calculated, and individuals with PI-HAT > 25% were eliminated from the group 2 to avoid potential confounding effects of shared sires.

The *MSTN* intron 1 SNP (rs397152648) genotype was investigated in related horses (group 1, progeny of a single sire) and unrelated horses (group 2) separately. All horses of group 1 were homozygous for the "C" allele (100%), and group 2 had the same result.

The SINE insertion was genotyped in all studied horses. PCR amplicons on 2% agarose gel were 600 bp fragments representing horses without SINE mutation (wild type or *N/N* genotype) (Figure 2). In fact, the SINE insertion was not present in any of the samples in both related (Figure 2a) and unrelated horses (Figure 2b), and all horses were "no insertion" for the SINE.

4 | Discussion

All related and unrelated Dareshouri horses were homozygous for the "C" allele. In a similar study conducted by Hill et al., 2010 on related and unrelated Thoroughbred horses to eliminate the effect of pedigree bias, the "C" allele was evident in both groups, and the results showed that the frequency of allele "C" was not significantly affected by the relationship between the half-sibs. But the purpose of choosing two groups in our study was to prove that the strong presence of "C" allele in Dareshouri horse breeds is not related to the genetic similarity of horses, because this frequency was observed in both studied groups.

The genotype of the rs397152648 SNP was completely monomorphic in this study, and the *C/C* genotype was the only genotype observed in this breed, and the absence of *C/T* and *T/T* genotypes was evident. This evidence was against the same studies in Thoroughbred, Quarter horse, Egyptian Arabian, Anglo-Arabian, Chinese and other horse breeds; these breeds are polymorphic for the genotype of rs397152648 SNP (Hill et al. 2010, Li et al. 2014, Pira et al. 2021). However, the frequency of the "C" allele is extremely high in Quarter horses and Thoroughbreds, and the *C/C* genotype was the most common genotype among Thoroughbred sprinters (0.51) and Quarter horses (0.83). This can be due to different selection pressures, the origin of the breed, the popular sire effect, and genetic drift in different breeds (Petersen et al. 2014). Afterward, the monomorphism of this locus in Dareshouri horses breed can be related to the extreme selection pressure for the phenotype because from the past until now, the breeders of this breed tended to choose more muscular horses with the idea that these horses are more suitable for use in the annual migration in mountainous areas, which has led to the loss of the genetic diversity and unusually high frequency of the rs397152648 SNP at the *MSTN* gene in the Dareshouri horse breed. In similar studies, the effect of selection pressure for the high frequency of the "C" allele and reduction of *MSTN* haplotype diversity has been reviewed in the Quarter horses and Thoroughbreds (Petersen et al. 2013, Petersen et al. 2014). In this way, strong selective pressures for speed in the racehorse applied during a short evolutionary time have led to intra-breed homogeneity and significant inter-breed variation in horses (Petersen et al. 2013).

Although in horses, the difference in skeletal muscle mass is not easily recognisable in appearance. Based on previous studies on the effect of the *C/C* genotype on body mass and best race performance in short-distance races, the C variant has been revealed to affect body composition in Thoroughbred in-training horses, and the muscle mass of horses with the *C/C* genotype is significantly greater than either *C/T* or *T/T* horses (Tozaki et al.

2011). Another study by Rooney et al., 2017 also confirmed this claim that the *C/C* genotype increases skeletal muscle mass by reducing *MSTN* gene expression.

In this way, these works had suggested that an intronic variant in *MSTN* (rs397152648 SNP) is related to better muscle mass ratio, and horses that were homozygous for the "C"allele had a greater amount of muscle mass than the other genotype (Tozaki et al. 2011, Rooney et al. 2017).

The estimation of muscle mass ratio in the Dareshouri horses and investigation of the relationship between muscle mass and C/C genotype may confirm the frequency of the "C" allele in this breed. It is better to measure body weight and wither height in trained horses to calculate the muscle mass ratio (Hill et al. 2010), but due to these horses not being trained for short-distance races, it is not possible to calculate this ratio in the present study.

Another variation surveyed in the *MSTN* gene in this study was SINE insertion. SINE insertions were genotyped in all horses with whole-genome SNP data. The SINE insertion was not present in any of the samples. The frequency of the allele without SINE insertion was 100% in both group 1 and group 2. Our findings are completely consistent with other studies that have reported SINE insertion only in Quarter horses and Thoroughbreds. The SINE is a new *MSTN* variant because of the low frequency of SINE (3.5%) in the reference genome (Santagostino et al. 2015). Researchers believe that this *MSTN* mutation was selected by breeders in order to obtain horses with special racing ability, and even though Quarter horse breeds were created from the crossing of Thoroughbred horses with other breeds, a higher frequency of SINE in the Quarter horses compared to the Thoroughbreds is more common (Santagostino et al. 2015). In research performed by Petersen et al. 2014 on SINE insertion in 14 breeds, only five breeds had the SINE insertion in the *MSTN* gene: the allele frequency of SINE was 0.73 and 0.48 in Quarter horses and Thoroughbreds, respectively, and this frequency was low in the other three breeds (0.11, 0.07 and 0.04). In another study on *MSTN* SINE insertion, all horses except Quarter horses and Thoroughbreds, and only 1 Uruguayan Creole horse, were homozygous for the wild type allele (Dall'Olio et al. 2014). In a study on Angola-Arabian horse breed, the SINE allele was not observed in horses with *C/T* genotype (Pira et al. 2021). In another research on five horse breeds (Quarter Horse, Andalusian, Lipizzaner, Norwegian Fjord and Icelandic Pony) and in Przewalski's horse, only in Quarter horses was the frequency of the SINE allele high (57%) and one breed had a low frequency of the SINE allele; in the other breeds and in Perzewalski's horse, the SINE insertion was not present (Santagostino et al. 2015).

Although some studies conducted on the effect of SINE insertion on *Myostatin* gene expression have reported that the SINE rather than the intron 1 SNP may affect the IIX muscle fibre percentage (Petersen et al. 2013, Petersen et al. 2014) and described that the correlation between intron 1 SNP and muscle mass and muscle fibre composition can be caused by linkage disequilibrium between SINE insertion and intron 1 SNP (Santagostino et al. 2015). Whereas the SINE MSTN gene was not present in any of the other studied breeds except Quarter horses and Thoroughbreds, this insertion might be a specific biomarker in these two specific breeds, not other horse breeds.

In addition, there are some racing horse breeds that compete at 1600 m, which are homozygous both for the allele without the insertion and the "C" allele (Dall'Olio et al. 2014). Additionally, a study on the presence of SINE insertion in the Turkmen speed breed (which cannot be referenced due to its publication in local journals) confirmed the absence of this variant in this racehorse breed. All this evidence confirmed that the SINE insertion is not a useful marker for association studies in all breeds.

According to the previous studies, there is a genomic region of about 2 Mb around *MSTN*, which is involved in the regulation of the transcripts related to the mitochondrial content, which is involved in the adaptation of horse muscle to training and exercise (Bryan et al. 2017), and there are multiple genetic factors, such as regulatory elements, that may further influence or modify the phenotype, which the effect of each genetic factor on racing performance may differ depending upon the genetic background within that factor (Petersen et al. 2014).

Because there are no studies on other gene loci and their relationship with the percentage of different muscle fibres, muscle mass and speed performance in racehorse breeds that do not have SINE insertion, the high frequency of allele "C" in these breeds cannot be completely considered unrelated or completely related to muscle mass and speed performance, and it is necessary to carry out complementary studies in this breed.

5 | Conclusion

Considering the fact that all the evidence confirmed the SINE insertion is not a useful marker for association studies in all breeds, furthermore, an intronic variant in *MSTN* (rs397152648 SNP) is related to better muscle mass ratio and best race performance. The presence of the rs397152648 SNP despite the absence of SINE insertion in the *Myostatin* gene in Dareshouri horses confirms that this breed has the genetic potential to cover the race distance in a shorter time.

Although the lack of other studies to check other *MSTN* haplotypic backgrounds, other influential gene loci, and regulatory factors affecting speed performance and survey percentage of different muscle fibres and measure muscle mass ratio, it is endorsed that other complementary studies in this breed need to prove this claim as to whether this breed may be a candidate for short-distance races.

Author Contributions

R.S.M designed and performed the experiments, analyzed the data, conducted the research, and wrote the main manuscript. F.M supported and supervised the project and contributed to manuscript revision. H.M supervised the project.

Ethics Statement

The authors used non-invasive methods to sample horses.

Conflicts of Interest

The authors declare no conflict of interest existing for this work.

Data Availability Statement

Data available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.70300

References

Binns, M. M., D. A. Boehler, and D. H. Lambert. 2010. "Identification of the Myostatin Locus (MSTN) as Having a Major Effect on Optimum Racing Distance in the Thoroughbred Horse in the USA." *Animal Genetics* 41: 154–158. https://doi.org/10.1111/j.1365-2052.2010.02126.x.

Bryan, K., B. A. McGivney, G. Farries, et al. 2017. "Equine Skeletal Muscle Adaptations to Exercise and Training: Evidence of Differential Regulation of Autophagosomal and Mitochondrial Components." *BMC Genomics [Electronic Resource]* 18, no. 1: 595. https://doi.org/10.1186/s12864-017-4007-9.

Budsuren, U., T. Ulaangerel, Y. Shen, et al. 2022. "MSTN Regulatory Network in Mongolian Horse Muscle Satellite Cells Revealed with miRNA Interference Technologies." *Genes* 13, no. 10: 1836–1836. https://doi.org/10.3390/genes13101836.

Dall'Olio, S., L. Fontanesi, L. Nanni Costa, M. Tassinari, L. Minieri, and A. Falaschini. 2010. "Analysis of HorseMyostatinGene and Identification of Single Nucleotide Polymorphisms in Breeds of Different Morphological Types." *Journal of Biomedicine and Biotechnology* 2010: 1–11. https://doi.org/10.1155/2010/542945.

Dall'Olio, S., E. Scotti, L. Fontanesi, and M. Tassinari. 2014. "Analysis of the 227 Bp Short Interspersed Nuclear Element (SINE) Insertion of the Promoter of the Myostatin (MSTN) Gene in Different Horse Breeds." *Veterinaria Italiana* 50, no. 3: 193–197. https://doi.org/10.12834/vetit.61.178.3.

Elkasrawy, M., and M. Hamrick. 2010. "Myostatin (GDF-8) as a Key Factor Linking Muscle Mass and Bone Structure. J Musculoskelet Neuronal Interact." *Journal of Musculoskeletal and Neuronal Interactions* 10, no. 1: 56–63.

Elliott, B., D. Renshaw, S. Getting, and R. Mackenzie. 2012. "The Central Role of Myostatin in Skeletal Muscle and Whole Body Homeostasis." *Acta Physiologica* 205, no. 3: 324–340. https://doi.org/10.1111/j.1748-1716.2012.02423.x.

Grobet, L., L. J. Royo Martin, D. Poncelet, et al. 1997. "A Deletion in the Bovine Myostatin Gene Causes the Double–Muscled Phenotype in Cattle." *Nature Genetics* 17, no. 1: 71–74. https://doi.org/10.1038/ng0997-71.

Hamrick, M. W., P. Arounleut, E. Kellum, M. Cain, D. Immel, and L.-F. Liang. 2010. "Recombinant Myostatin (GDF-8) Propeptide Enhances the Repair and Regeneration of both Muscle and Bone in a Model of Deep Penetrant Musculoskeletal Injury." *The Journal of Trauma: Injury, Infection, and Critical Care* 69, no. 3: 579–583. https://doi.org/10.1097/ta.0b013e3181c451f4.

Hill, E. W., J. Gu, S. S. Eivers, et al. 2010. "A Sequence Polymorphism in MSTN Predicts Sprinting Ability and Racing Stamina in Thoroughbred Horses." *PLoS ONE* 5, no. 1: e8645. https://doi.org/10.1371/journal.pone.0008645.

Kellum, E., H. Starr, P. Arounleut, et al. 2009. "Myostatin (GDF-8) Deficiency Increases Fracture Callus Size, Sox-5 Expression, and Callus Bone Volume." *Bone* 44, no. 1: 17–23. https://doi.org/10.1016/j.bone.2008.08.126.

Li, R., D.-H. Liu, C.-N. Cao, et al. 2014. "Single Nucleotide Polymorphisms of Myostatin Gene in Chinese Domestic Horses." *Gene* 538, no. 1: 150–154. https://doi.org/10.1016/j.gene.2013.12.027.

McGivney, B. A., J. A. Browne, R. G. Fonseca, et al. 2012. "MSTNgenotypes in Thoroughbred Horses Influence Skeletal Muscle Gene Expression and Racetrack Performance." *Animal Genetics* 43, no. 6: 810–812. https://doi.org/10.1111/j.1365-2052.2012.02329.x.

McPherron, A. C., and S.-J. Lee. 1997. "Double Muscling in Cattle due to Mutations in the Myostatin Gene." *Proceedings of the National Academy of Sciences* 94, no. 23: 12457–12461. https://doi.org/10.1073/pnas.94.23.12457.

Mosher, D. S., P. Quignon, C. D. Bustamante, et al. 2005. "A Mutation in the Myostatin Gene Increases Muscle Mass and Enhances Racing Performance in Heterozygote Dogs." *PLoS Genetics* 3, no. 5: e79. https://doi.org/10.1371/journal.pgen.0030079.

Peters, S., J. Wilson, and J. Boyette. 2020. "Differential Expression of IGF1, IGFBP5, MSTN, and MYH1 across Different Age Classes in American Quarter Horses." *Journal of Equine Veterinary Science* 94: 103226. https://doi.org/10.1016/j.jevs.2020.103226.

Petersen, J. L., J. R. Mickelson, A. K. Rendahl, et al. 2013. "Genome-Wide Analysis Reveals Selection for Important Traits in Domestic Horse Breeds." *PLoS Genetics* 9, no. 1: e1003211. https://doi.org/10.1371/journal.pgen.1003211.

Petersen, J. L., S. J. Valberg, J. R. Mickelson, and M. E. McCue. 2014. "Haplotype Diversity in the Equinemyostatingene with Focus on Variants Associated with Race Distance Propensity and Muscle Fibre Type Proportions." *Animal Genetics* 45, no. 6: 827–835. https://doi.org/10.1111/age.12205.

Pira, E., G. M. Vacca, M. L. Dettori, et al. 2021. "Polymorphisms at Myostatin Gene (MSTN) and the Associations with Sport Performances in Anglo-Arabian Racehorses." *Animals* 11, no. 4: 964. https://doi.org/10.3390/ani11040964.

Rooney, M. F., E. W. Hill, V. P. Kelly, and R. K. Porter. 2018. "The 'Speed Gene' effect of Myostatin Arises in Thoroughbred Horses due to a Promoter Proximal SINE Insertion." *PLoS ONE* 13, no. 10: e0205664. https://doi.org/10.1371/journal.pone.0205664.

Rooney, M. F., R. K. Porter, L. M. Katz, and E. W. Hill. 2017. "Skeletal Muscle Mitochondrial Bioenergetics and Associations with Myostatin Genotypes in the Thoroughbred Horse." *PLoS ONE* 12, no. 11: e0186247. https://doi.org/10.1371/journal.pone.0186247.

Santagostino, M., L. Khoriauli, R. Gamba, et al. 2015. "Genome-Wide Evolutionary and Functional Analysis of the Equine Repetitive Element 1: An Insertion in the Myostatin Promoter Affects Gene Expression." *BMC Genet* 16, no. 1: https://doi.org/10.1186/s12863-015-0281-1.

Schuelke, M., K. R. Wagner, L. E. Stolz, et al. 2004. "Myostatin Mutation Associated with Gross Muscle Hypertrophy in a Child." *New England Journal of Medicine* 350, no. 26: 2682–2688. https://doi.org/10.1056/nejmoa040933.

Stinckens, A., M. Georges, and N. Buys. 2010. "Mutations in the Myostatin Gene Leading to Hypermuscularity in Mammals: Indications for a Similar Mechanism in Fish?" *Animal Genetics* 42, no. 3: 229–234. https://doi.org/10.1111/j.1365-2052.2010.02144.x.

Thomas, M., B. Langley, C. Berry, et al. 2000. "Myostatin, a Negative Regulator of Muscle Growth, Functions by Inhibiting Myoblast Proliferation." *Journal of Biological Chemistry* 275, no. 51: 40235–40243. https://doi.org/10.1074/jbc.m004356200.

Tozaki, T., T. Miyake, H. Kakoi, et al. 2010. "A Genome-Wide Association Study for Racing Performances in Thoroughbreds Clarifies a Candidate Region near the MSTN Gene." *Animal Genetics* 41: 28–35. https://doi.org/10.1111/j.1365-2052.2010.02095.x.

Tozaki, T., F. Sato, E. W. Hill, et al. 2011. "Sequence Variants at the Myostatin Gene Locus Influence the Body Composition of Thoroughbred Horses." *Journal of Veterinary Medical Science* 73, no. 12: 1617–1624. https://doi.org/10.1292/jvms.11-0295.