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Original Research

MSTN, *CKM*, and *DMRT3* Gene Variants in Different Lines of Quarter Horses

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ABSTRACT

Single nucleotide polymorphisms (SNPs) in the equine myostatin (*MSTN*) (g.66493737C>T) and creatine kinase muscle (*CKM*) (g.22999655C>A) genes have been associated with optimum racing distance and muscle development and racing performance in Thoroughbred horses, respectively. Considering that, since its formation, the Quarter Horse breed has received important genetic influence from the English breed, the genes cited become important candidates for athletic performance in the racing line of the American breed. An SNP in the equine doublesex and mab-3-related transcription factor 3 (*DMRT3*) gene (g.22999655C>A) has been described, which is responsible for the gait phenotype in homozygous individuals. Using a sample of 296 Quarter Horses of the racing line and 68 animals of the cutting line, the objective of this study was to compare the frequencies of the three SNPs cited above between a random subsample of animals of the cutting line (n = 20) and animals with extreme phenotypes for racing performance (n = 20 per extreme phenotype). The *MSTN* SNP showed practically no variation, with the observation of only one heterozygous animal (CT) in the cutting line, suggesting that this gene has been under great selective pressure within the racing segment. The *CKM* gene variant studied was found to be polymorphic, but no significant associates were observed between its alleles and the different lines or groups. Two animals carrying the CA heterozygous *DMRT3* genotype were identified in the group with poor racing performance and one in the cutting line, indicating that this variant can be a limiting factor for the development of greater speeds.

1. Introduction

The Quarter Horse breed originated in the beginning of the 17th century as a result of crossings between breeds brought by the English settlers to America and wild horses introduced by Spanish colonizers [1]. As a result of different selection objectives, different segments or lines of the breed currently exist, including the cutting line and racing line. Animals of the racing line show a better performance in short-distance races than any other line or breed and are the fastest horses and one of the fastest animals in the world [1]. The cutting line is destined for functional tests, exploring skills such as agility and obedience, traits that are important for cattle management in the field [2].

As observed for humans [3], athletic performance in horses is likely to be influenced by a large number of genes. However, only few genetic variants have so far been related to this trait exclusively in Thoroughbred animals, including single nucleotide polymorphisms (SNPs) in the myostatin

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gene (*MSTN*) [4–8] and the creatine kinase muscle gene (*CKM*) [9]. The *MSTN* gene is expressed in skeletal muscle tissue and acts as a negative regulator of muscle mass growth. Several mutations or polymorphisms have been identified in the *MSTN* gene of cattle [10–12], sheep [13], mice [14], and humans [15], which result in phenotypes of hyperplasia and muscle hypertrophy. The *CKM* gene encodes a muscle isoenzyme of creatine kinase found exclusively in striated muscle, which is involved in important energy processes in the cell, especially the generation of adenosine triphosphate during the first seconds of intense exercise [16]. Recently, an SNP in the equine doublesex and mab-3-related transcription factor 3 (*DMRT3*) gene has shown a highly significant association with the type of gait. This alteration causes significant changes in the motor coordination of trot and gallop (racing) movements [17].

In view of the role of the genes cited in skeletal muscle and nervous system physiology, the influence of Thoroughbreds on the formation of Quarter Horses and the knowledge that the effect of DNA polymorphisms on phenotypes are intrinsic parameters of each line or breed in a certain environment; the objective of the present study was to compare the frequencies of the *MSTN* g.66493737C>T, *CKM* g.15884567A>G, and *DMRT3* g.22999655C>A SNPs between the racing and cutting lines of Quarter Horses and between animals with extreme phenotypes for racing performance. The results of this study are expected to improve the understanding of the importance of the protein products of the *MSTN*, *CKM*, and *DMRT3* genes for performance-related physiology in horses. Furthermore, the results obtained, through the use of molecular markers, may contribute to the selection of superior Quarter Horses for this trait.

2. Material and Methods

2.1. Animals, Blood Collection, and Performance Data

For this study, 364 Quarter Horses of both sexes, born between 1982 and 2011 and registered at the Brazilian Association of Quarter Horse Breeders, were used. Of these, 296 animals were of the racing line and 68 of the cutting line. The racing animals, including 67 males and 229 females born to 95 stallions and 240 mares, were housed at the Sorocaba Jockey Club and on 14 other properties in the state of São Paulo, Brazil. The cutting horses, including 26 males and 42 females born to 44 stallions and 64 mares, were housed on three properties in the state of São Paulo. Blood was collected on the horse farms in the state of São Paulo and at the Sorocaba Jockey Club. The use of full-sibs in the two lines was not practiced.

Whole blood (5 mL) was collected from each animal by puncture of the left jugular vein in the neck region into vacuum tubes containing 7.5 mg EDTA. Performance data (sped index [SI]) were obtained from the Department of Statistics of the Sorocaba Jockey Club and from the Equibase online database [18]. The SI of several years was available for most of the animals used, and the mean SI was thus calculated. However, only the maximum SI (the best score of the animal obtained during his entire life) was available for other animals. Because the mean SI showed a high correlation with maximum SI ($r = 0.8762$), the latter was used for analysis to prevent the loss of animals. According to Evans [19], the SI was created for Quarter Horse races to permit the comparison of performance between animals under different conditions (distances, racetrack, climate, and country). Details on SI calculation have been described previously by Meira et al [20].

All animal procedures were performed according to Brazilian guidelines of animal well-being (Protocol No. 204/2012-CEUA issued by the Ethics Committee on Animal Use of the School of Veterinary Medicine and Animal Science, UNESP, Botucatu, São Paulo, Brazil).

2.2. Determination of Extreme Groups and Genotyping of Animals

For the formation of groups of 20 animals with lower and higher SIs, the phenotypes of 296 animals of the racing line were adjusted for the systematic effects of environment (fixed effects), sex, the interaction between race track (1–14) and distance (228, 275, 301, 320, 365, 402, and 502 m), and the interaction between year of race (1988–2013) and age of animal at race (2, 3, and 4 years). The significance of the effect of sex and of the interactions was tested using the PROC GLM procedure of the SAS v.9.1 program [21].

DNA was extracted from a subsample of 60 animals for subsequent genotyping of the polymorphisms of interest, including 40 animals of the racing line (20 per extreme phenotype) and 20 animals of the cutting line (extracted randomly from the total sample of 68 animals) using the Illustra Blood GenomicPrep Mini Spin Kit (GE Healthcare, USA), according to the manufacturer instructions.

For analysis of the target SNPs of the *MSTN* and *CKM* genes, the polymerase chain reaction (PCR) products, including their flanking regions (463 and 556 bp, respectively), obtained with the primers described in Table 1, were sequenced. The sequences were generated with the ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems, USA) and analyzed using the Phred/Phrap/Consed package [22].

The g.22999655C>A SNP of the equine *DMRT3* gene was analyzed by PCR–restriction fragment length polymorphism (RFLP), in which a fragment of 470 bp was amplified and digested with the restriction enzyme *Dde*I

Table 1
Sequences of the primers used for genotyping of the SNPs in the candidate genes analyzed in the sample of animals studied.

Gene/ID	Primer 5′-3′	Genotyping Method
CKM-F	GCATAGGAATATATGCGAATCCT	Sequencing
CKM-R	GAAGAGCATGACGGAGCAG	
DMRT3-F	GGGAACAGAATCACCTCCTG	PCR–RFLP
DMRT3-R	CGACTGGTTTCTTGCCAAAG	
MSTN-F	TATTCTTCTTGGGAGGGAGGACTACT	Sequencing
MSTN-R	GCAAGTAATTAGCACAAAAATTTGAATG	

Abbreviations: *CKM*, creatine kinase muscle; *DMRT3*, doublesex and mab-3-related transcription factor 3; *MSTN*, myostatin; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SNP, single nucleotide polymorphism.