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Original Research

Comparison of Sequence Variants in the *PDK4* and *COX4I2* Genes Between Racing and Cutting Lines of Quarter Horses and Associations With the Speed Index

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ABSTRACT

Different selection objectives within the Quarter Horse breed led to the formation of groups with distinct skills, including the racing and cutting lines. With a smaller population size in Brazil, but of great economic representativeness, the racing line is characterized by animals that can reach high speeds over short distances and within a short period of time. The cutting line is destined for functional tests, exploring skills such as agility and obedience. Although the athletic performance of horses is likely to be influenced by a large number of genes, few genetic variants have so far been related to this trait and this was done exclusively in Thoroughbreds, including the g.38973231G>A single-nucleotide polymorphism in the *PDK4* gene and the g.22684390C>T single-nucleotide polymorphism in the *COX4I2* gene. The results of the present study demonstrate the presence of polymorphic *PDK4* and *COX4I2* genes in Quarter Horses. The analysis of 296 racing animals and 68 cutting animals revealed significant differences in allele and genotype frequencies between the two lines. The same was not observed when these frequencies were compared between extreme racing performance phenotypes. There were also no significant associations between alleles of the two polymorphisms and the speed index. These results suggest that the alleles of the *PDK4* and *COX4I2* genes, which are related to better racecourse performance in Thoroughbreds, are probably associated with beneficial adaptations in aerobic metabolism and therefore play secondary roles in sprint racing performance in Quarter Horses, which is mainly anaerobic.

1. Introduction

During the formation of the Quarter Horse breed, animals were selected with different objectives which resulted in groups with different skills or abilities, such as the racing and cutting lines [1]. Quarter Horse racehorses 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. The Quarter Horse can reach a speed of up to 88 km/hr and can sprint the quarter mile (approximately 402 m) from a standing position in less than 21 seconds [2]. The cutting line is destined for functional tests, exploring skills such as agility and obedience, which are important for cattle management in the field. A cutting horse should be able to perceive and anticipate the movements of cattle to be a good sorting horse [3].

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The athletic potential of mammals is influenced by the complex interrelationship between a set of genes and environmental factors [4,5]. The contribution of genetics to athletic potential is well documented in humans, in which more than 220 genes have been described [6]. Although the athletic performance of horses is probably also influenced by a large number of genes, few genetic variants have so far been related to this trait and this was done exclusively in Thoroughbreds. These variants include single-nucleotide polymorphisms (SNPs) in the pyruvate dehydrogenase kinase, isozyme 4 (*PDK4*: mapped at equine chromosome 22—ECA22) gene [7,8] and in the cytochrome c oxidase (COX), subunit 4, isoform 2 (*COX4I2*: mapped at equine chromosome 4—ECA4) gene [9].

The regulation of glucose utilization is tightly controlled by the uptake of glucose by their transporters, the rate of glycolytic flux, and the conversion of pyruvate to acetyl-CoA in the mitochondria through the catalytic function of the pyruvate dehydrogenase complex (PDC). The critical rate-limiting step of glucose oxidation is the regulation of PDC assembly, which is controlled by pyruvate dehydrogenase kinase. Pyruvate dehydrogenase kinase blocks the formation of the PDC, which results in the beta-oxidation of fatty acids to acetyl-CoA as the substrate for oxidative phosphorylation. The oxidation of fatty acids is highly efficient in the generation of adenosine triphosphate (ATP) and is controlled by the expression of *PDK4* in skeletal muscle during and after exercise [10].

Cytochrome c oxidase is a multisubunit enzyme (complex IV) which catalyzes the transfer of electrons from reduced cytochrome c to oxygen during mitochondrial respiration. Cytochrome c oxidase complex IV, which is encoded by nuclear DNA, is responsible for the regulation and assembly of mitochondrially encoded subunits on the mitochondrial membrane and has been associated with mitochondrial volume. Cytochrome c oxidase complex IV consists of two isoforms (COX4-1 and COX4-2) that are encoded by the *COX4I1* and *COX4I2* genes. The two genes are differentially regulated in normoxic (normal oxygen) and hypoxic (lack of oxygen) environments. The *COX4I1* gene is preferentially transcribed in normal oxygen environments. In limited oxygen environments, the master regulator of the response to hypoxia, hypoxia inducible factor 1, activates the transcription of *COX4I2* and of the mitochondrial *LON* gene, which inhibits the expression of *COX4I1* [11].

In view of the role of the genes described in the physiology of skeletal muscle and considering that the effects of DNA polymorphisms on phenotypes are intrinsic parameters of each line or breed in a given environment, the objectives of the present study were (1) to compare the frequencies of the *PDK4* g.38973231G>A and *COX4I2* g.22684390C>T SNPs (EquCab 2.0, [12]) between the racing and cutting lines of Quarter Horses and between animals with extreme racing performance phenotypes and (2) to perform an association analysis of these polymorphisms with the speed index (SI), a quantitative trait indicative of the racing performance of Quarter Horses. The results of this study may contribute to marker-assisted selection of Quarter Horses for better racecourse performance.

2. Materials and Methods

2.1. Animals and Performance Data

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).

For this study, 364 Quarter Horses of both sexes, born between 1982 and 2011 and registered at the Brazilian Association of Quarter Horse Breeders (Associação Brasileira de Criadores de Cavalo Quarto de Milha), 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 presence of full-sibs in the two lines was avoided.

Performance data were obtained from the Department of Statistics of the Sorocaba Jockey Club and from the Equibase online database [13]. The performance record is given by the maximum SI obtained along the competition history of each animal. According to Evans [14], the SI was created for Quarter Horse races to permit the comparison of performances between animals under different conditions (distances, racetrack, climate, and country). Every year each racetrack creates its own SI table, which is derived from the average of three wins (top three times) for each of the last three consecutive years in each distance, and the average value of those nine times is equivalent to an SI of 100, creating a scale. Therefore, SI points are integers and vary with time (longer times lead to lower indices and vice versa), the level of hundredths of a second, and are adjusted by the distance traveled in the race. 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 was available for other animals. Because the mean SI showed a high correlation with maximum SI ($r = 0.8762$), the latter was used to prevent the loss of performance data. The mean and standard deviation of maximum SI considering 267 animals of the racing line with the data were 95.78 ± 7.80.

2.2. Blood Collection, DNA Extraction, and Genotyping of Animals

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. DNA was extracted using the Illustra Blood GenomicPrep Mini Spin Kit (GE Healthcare, USA) according to the manufacturer instructions.

For determination of the allele and genotype frequencies and for association analysis of the *PDK4* and *COX4I2* gene polymorphisms with performance, 364 Quarter Horses (racing line, n = 296; cutting line, n = 68)