Livestock Science 216 (2018) 160–164

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Polymorphisms in the *LASP1* gene allow selection for smaller stature in ponies

Aylton Bartholazzi Junior*, Celia Raquel Quirino, Wilder Hernando Ortiz Vega, Miguel Alejandro Silva Rua, Caroline Marçal Gomes David, Júlia Gazzoni Jardim

Laboratory for Animal Breeding and Genetic Improvement, Norte Fluminense State University, Campos dos Goytacazes, RJ CEP: 28013-602, Brazil

ARTICLE INFO

Keywords:
Genetic marker
Genomic selection
Miniature horse
Regulatory elements
Variants

ABSTRACT

The LIM and SH3 protein 1 (*LASP1*) and the ligand dependent nuclear receptor corepressor-like (*LCORL*) genes were selected as candidates in this study of growth and height of equines of the Brazilian Pony breed. Until now, no genomic studies on the morphometry of Brazilian ponies have been reported in the literature.

Both genes are correlated with osteogenesis and transcription factor TFIID of genes involved in skeletal growth, respectively. The objective of this study was to analyze two height associated mutations in *LASP1* and *LCORL* genes and their associations with equine morphometry. This is the first study that genotyped these known variants in the Brazilian Pony breed. The DNA was extracted from hair follicles and the two variants were amplified by PCR. The genotyping was performed by RFLP markers through restriction enzyme cleavage; the samples were submitted to capillary electrophoresis for allelic discrimination by size; and the polymorphism was identified and associated with the morphometric measures of the animals. Regarding the *LASP1* gene, the ponies with genotypes G/G and G/A presented the smallest measures for head length, neck length, withers height, croup height and body length. Mutation analysis [T > C] of the *LCORL* gene identified the T/T genotype in all ponies except one individual of the control group, with shoulder height of 1.69 m, which presented the C/C genotype, confirming the association of this gene with the height of horses of the Brazilian Pony breed. The stature and morphometry of horses of this breed were influenced by variants of the *LASP1* gene.

1. Introduction

In general, the selection in horses is based on evaluation of subjective traits such as running speed or awards at shows (Costa et al., 2005), with use of only a few studs and mares for reproduction. However, breeders' associations, especially of ponies, use thresholds of morphological measures of withers height in the records of the animals (ABCCPONEI, 2018), considering the importance of size and conformation in the evaluation of these animals.

The Brazilian Pony breed is descended from Shetland of Scotland, Falabella of Argentina, and ponies from Paraguay and Uruguay. As a breed standard, their height cannot exceed 1.00 m in males and 1.10 m in females (ABCCPÔNEI, 2018). In Brazil, this breed is of great importance, mainly used for recreation, teaching riding to children and equine therapy programs (Rua et al., 2016).

In order to increase the efficiency of traditional selection for economic traits in animals, such as size, athletic performance, gait and musculature, beadchip genotyping is currently used for selection of different species (Petersen et al., 2013). However, this type of technology is not widely used for equines.

Several studies of human stature have identified more than 200 loci with effect on height (Allen et al., 2010; Okada et al., 2010). Genome-wide association studies in cattle (Pryce et al., 2011; Lindholm-Perry et al., 2013; Xu et al., 2014), horses (Makvandi-Nejad et al., 2012; Metzger et al., 2013; Tetens et al., 2013; Boyko et al., 2014) dogs (Vaysse et al., 2011), have mapped many genes previously associated with height in humans, strongly suggesting that polymorphic regions affect various species.

Some genes have been associated with body growth in horses. Makvandi-Nejad et al. (2012) performed a study of genome wide association and identified polymorphisms in the genes *LASP1, LCORL, HMGA2* and *ZFAT*, responsible for affecting stature of different equine breeds, which explained 83% of the variation in the studied animals. Other studies reporting genes associated with height are Frischknecht et al. (2015), who identified the *HMGA2* gene, and Al Abri et al. (2016), who observed through a genome-wide association study the presence of the *ANKRD1* gene.

The LIM and SH3 protein 1 (*LASP1*) is a candidate gene of stature in

* Corresponding author.
E-mail address: junior_barth@hotmail.com (A. Bartholazzi Junior).

https://doi.org/10.1016/j.livsci.2018.07.015
Received 5 September 2017; Received in revised form 23 July 2018; Accepted 27 July 2018

animals, because its expression level affects the formation of cartilaginous tissue, osteogenic differentiation and cell migration (Hermann-Kleiter et al., 2009; Hu et al., 2014; Lin et al., 2004). Similarly, the ligand dependent nuclear receptor corepressor-like gene (*LCORL*) has been associated with height of horses by various authors (Makvandi-Nejad et al., 2012; Boyko et al., 2014), possibly linked to a transcription factor associated with genes involved in the development of bony skeleton (Metzger et al., 2013).

Until now, no genomic studies on morphometry of the Brazilian Pony breed have been published. Due to the global economic importance of early selection of stature traits in pony breeds and the lack of existing genomic information, the objective of this work was to analyze single nucleotide polymorphisms (SNP) on the *LASP1* and *LCORL* genes and their associations with morphometry Brazilian ponies. In addition, this is the first study that genotyped these known variants in this breed.

2. Material and methods

This study was approved by the Ethics Committee on Animal Experimentation of Norte Fluminense State University (UENF Protocol no. 245) in accordance with the Sociedade Brasileira de Ciência de Animaisde Laboratório/Colégio Brasileiro de Experimentação Animal (SBCAL/COBEA).

2.1. Collection of DNA samples and morphometric measurements

Samples of hair from the base of the tail were collected from 124 horses (age average equal or higher than 3 years) for genotyping. Of these, 115 animals (27 males and 88 females) were Brazilian ponies, from two farms, and 9 equines as controls from different genetic groups (Mangalarga Marchador, Mangalarga Paulista, Pampa, Lusitano, Arabian, Lusitano × Arabian, Quarter Horse, Paint Horse and Percheron). All horses were samples in the state of Rio de Janeiro, Brazil.

The morphometric measurements of each animal were obtained using a zoometric hypsometer and non-elastic tape measure. During measurements, the animals were on a firm and flat surface, with all four hooves on the ground. The measurements are depicted in Fig. 1.

2.2. DNA extraction and PCR conditions

The DNA was extracted from hair follicles collected from tails, using a NucleoSpin tissue kit (Macherey-Nagel, Düren, Germany). The DNA concentration was measured by spectrophotometer (NanoDrop®) and the extracted DNA was diluted to a final concentration of 10 ng/uL for each sample.

Table 1
Primers for fragment amplification of *LCORL* and *LASP1* genes.

Loci	PrimerA 5′–3′	PrimerB 5′–3′	Ta
LCORL	Gtcaaagccagaggtggagag	Acctctggctttgaccgg	65°C
LASP1	ggatgactggcctaagcttgg	gtgacagcactggggcact	66°C

Ta = Annealing temperature.

PCR was performed for gene-specific variants *LCORL* and *LASP1* following the information contained in Table 1. The primers of the regions of interest were modeled with the Gene Runner® software and evaluated by the Primer Express® software.

The final volume of PCR was 20 µl per reaction for each locus, using buffer 1× [10 mM Tris–HCl (add Mg^{2+})], 0.5 mM of Promega® dNTP mix, 1 U of Promega® *Taq* DNA polymerase, 0.5 µM of each primer – Invitrogen® (Table 1), deionized water and 20 ng of content obtained from DNA extraction. PCR reaction was performed in an Applied Veriti® 96-well thermocycler.

Aliquots (3 µl) of amplified samples were subjected to polyacrylamide gel electrophoresis (Sigma-Aldrich, St. Louis, MO, USA) after non-denaturing at 8%. Along with the samples, molecular weight standards were applied (Promega® 100 bp DNA ladder) for confirmation of amplified fragments. After confirmation of the amplification by visualization on the polyacrylamide gel, the samples were subjected to restriction enzyme cleavage.

2.3. Genotyping for LCORL and LASP1 genes

The amplified DNA was genotyped for mutations of the *LCORL* gene (T > C, chromosome: 3, rs68603064) and *LASP1* gene (G > A, chromosome: 11, rs68876319), as identified by Makvandi-Nejad et al. (2012). Genotyping was performed by RFLP through cleavage by restriction enzymes for *LCORL* variant (BsrI) and for *LASP1* variant (MwoI) chosen using the NEBcutter® V2.0 software (Table 2).

All cleavage reactions with restriction enzyme were carried out using 20 µl as final volume: 5 U of enzyme, 4 µl of PCR product and deionized water. A specific buffer (1×) was used for each enzyme and a negative control was included in all reactions. Samples were placed in the thermocycler (Applied Veriti® 96-well) according to the specific incubation temperature of each enzyme (Table 2). The product of the reactions with restriction enzymes was subjected to capillary electrophoresis using a Fragment Analyzer™ (Advanced Analytical) for allelic discrimination by size and identification of the polymorphism.

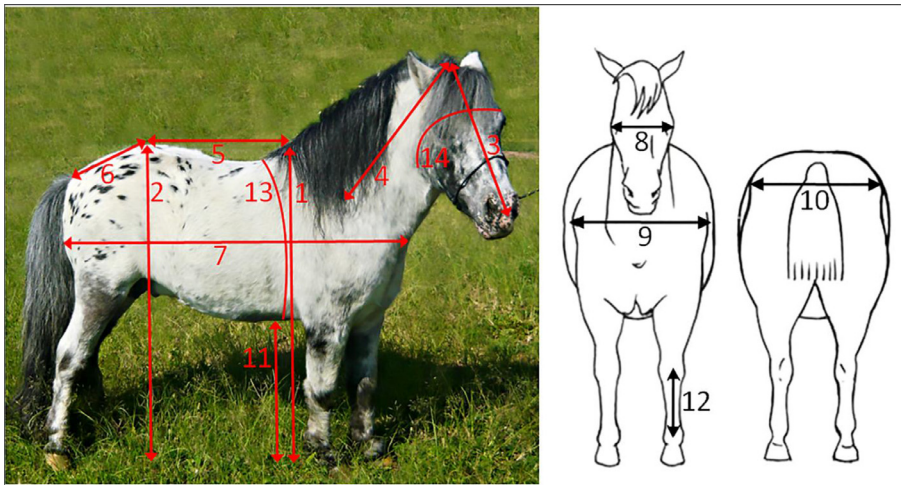

Fig. 1. Representation of the linear measurements of horses of the Brazilian Pony breed. 1. Wither height, 2. Croup height, 3. Head length, 4. Neck length, 5. Backloin length, 6. Croup length, 7. Body length, 8. Head width, 9. Bi-scapular width, 10. Rump width, 11. Thorax height, 12. Front cannon length, 13. Thoracic perimeter and 14. Head diameter (Adapted from Bartholazzi Junior et al., 2017).