# *ACTN3* gene variants as potential phenotype and performance biomarkers in Brazilian sport horses training for eventing in a tropical climate

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## Abstract

The aim of this study was to look for mutations in the equine *ACTN3* gene and to identify sequence variants that might be associated with the phenotype and performance of Brazilian sport horses training for events in a tropical climate. Among 17 such horses direct DNA sequencing and mutation analysis of the exon 15 and the intron–exon boundaries of *ACTN3* revealed 2 new sequence variants in the *ACTN3* intron 14–15, designated c.1681–86G > A and c.1681–129delA. Wild-type/deletion heterozygotes (A/del) had a lower mean subcutaneous fat layer in the region of the gluteus medius, as measured by ultrasonography, than the del/del homozygotes; the correlation was significant ($P = 0.017$). This single base-pair deletion in *ACTN3* intron 14–15 may have resulted in metabolic changes that led to increased deposition of body fat in the homozygous state. However, neither sequence variant was correlated with the time to fatigue in a test on a high-speed treadmill with an incremental-speed protocol.

## Résumé

*Le but de la présente étude était de vérifier pour la présence de mutations dans le gène* ACTN3 *équin et d'identifier des variants de séquence qui pourraient être associés avec le phénotype et la performance de chevaux de sport brésiliens qui s'entraînent pour des concours dans un climat tropical. Parmi 17 chevaux qui correspondent à ces critères, le séquençage direct de l'ADN et l'analyse de mutation de l'exon 15 et des frontières de l'intron-exon d'*ACTN3 *a révélé deux nouveaux variants de séquence dans l'intron 14–15 d'*ACTN3*, désigné c.1681–86G > A et c.1681–129delA. Chez les hétérozygotes type-sauvage/délétion (A/del) la moyenne de l'épaisseur de la couche de gras sous-cutané dans la région du gluteus medius était plus petite, telle que mesurée par échographie, que celle des homozygotes del/del; la corrélation était significative (*P = *0,017). Cette délétion unique de paire de bases dans l'intron 14–15 d'*ACTN3 *pourrait avoir résulté dans des changements métaboliques qui auraient mené à une augmentation du dépôt de gras chez les homozygotes. Toutefois, aucun des variants de séquence n'était corrélé avec le temps de fatigue dans un test sur un tapis-roulant à haute vitesse avec un protocole d'augmentation de vitesse.*

*(Traduit par Docteur Serge Messier)*

Alpha-actinin-3 (ACTN3) is an actin-binding protein specific to fast-twitch muscle fibers that influences power generation in high-speed activities (1). A deficiency of ACTN3 results in a shift in muscle metabolism from the glycolytic pathway toward the oxidative pathway (2–5). Low *ACTN3* expression is related to a reduction in the diameter of fast-twitch fibers, increased activity of aerobic enzymes, a change in contractile properties, and improved recovery from fatigue (1,2,4,6). The aim of this study was to look for mutations in the exon 15 and the intron–exon boundaries of equine *ACTN3* and to identify sequence variants that might be associated with phenotype and performance of Brazilian sport horses training for events in a tropical climate. The study procedures were approved by the Ethics Committee for Use of Animals of the Universidade Federal Fluminense, Niterói, Rio de Janeiro, Brazil (protocol 276/2013).

Of the 17 Brazilian sport horses in training for such events that were selected for genotyping, 6 were mares and 11 were geldings. Their ages ranged from 4 to 10 y, with a mean of 7.59 ± 1.87 [standard deviation (SD)] y. They were housed in masonry stalls 4 × 4 m with free access to water, and 3 times a day they were fed "coast-cross" hay *(Cynodon dactylon* L. Pers.*)* and commercial concentrate (1% body weight) containing guaranteed levels of the following:

maximums for:

- fibrous matter — 150 g/kg (15%);
- acid detergent fiber — 180 g/kg (18%);
- mineral matter — 120 g/kg (12%); and
- calcium — 20 g/kg (2%).

minimums for:

- crude protein — 120 g/kg (12%);

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ethereal extract — 40 g/kg (4%);
calcium — 15 g/kg (1.5%);
phosphorus — 5000 mg/kg (0.5%);
digestible energy — 2700 kcal/kg;
methionine — 1800 mg/kg;
lysine — 4800 mg/kg;
vitamin A — IU/kg;
vitamin D3 — 1000 IU/kg;
vitamin E — 100 IU/kg;
vitamin B1 — 5 mg/kg;
vitamin B2 — 4 mg/kg;
copper — 20 mg/kg;
iodine — 0.5 mg/kg;
manganese — 64 mg/kg;
selenium — 0.2 mg/kg;
cobalt — 0.14 mg/kg; and
zinc — 80 mg/kg.

During the year of the study the average minimum and maximum temperatures in Rio de Janeiro were 21.7°C and 30.3°C according to the Brazilian National Institute of Meteorology; the mean relative humidity was 72.67% (7).

Mutation analysis of the *ACTN3* gene regions of interest was by direct DNA sequencing, according to Mata et al (8). The equine *ACTN3* reference genomic sequence used in the analysis was retrieved from GenBank [National Center for Biotechnology Information (NCBI), Bethesda, Maryland, USA] with the accession number NC_00009155.2.

The thickness of the subcutaneous fat layer was measured in the region of the equine muscle gluteus medius by ultrasonography. Effort was determined with an incremental speed test on a high-speed treadmill, the horses exercising at 8.0 m/s until fatigued (Table I). The treadmill inclination simulated the rider's weight.

The significance of associations between quantitative variables was determined with Pearson's correlation coefficient and significance testing. Differences in qualitative variables between independent groups were compared by means of Fisher's exact test. Between-group differences in the value of quantitative variables were compared by means of the nonparametric Mann–Whitney test. A 5% significance level was used in all tests. Statistical analysis was done with the use of SPSS software, Version 17 (Unicom, Mission Hills, California, USA).

Mutation analysis of the equine *ACTN3* exon 15 and intron–exon boundaries revealed 2 new sequence variants. One variant, designated c.1681–86G > A (g.26522074G > A; NCBI ss2019497321), was observed in the *ACTN3* intron 14–15 of 9 (53%) of the 17 horses; 8 horses (47%) had the wild-type homozygous genotype (GG), 7 (41%) had the heterozygous genotype (GA), and 2 (12%) were mutant homozygotes (AA). The other variant, c.1681–129delA (g.26522031delA; NCBI ss2019497320), was also identified in the equine *ACTN3* intron 14–15; none of the 17 horses had the wild-type homozygous genotype (AA), 5 (29%) were heterozygotes (A/del), and 12 (70%) were mutant homozygotes (del/del). Even though the *ACTN3* exon 15 sequence is conserved in humans and horses, the p.R577X variant associated with physical performance in humans was not observed in the 17 horses studied. All the genotype frequencies identified in this study showed Hardy–Weinberg equilibrium, demonstrating that the analyzed genotypes were randomly sampled from the general population.

**Table I. Protocol of an incremental speed test on a high-speed treadmill for 17 Brazilian sport horses training for events in a tropical climate.**

| Phase | Time (min) | Velocity (m/s) | Gait | Incline (%) |
|---|---|---|---|---|
| Warm-up | 2 | 1.7 | Walk | 0 |
| Warm-up | 4 | 4.0 | Trot | 0 |
| Warm-up | 4 | 4.0 | Trot | 3 |
| Gallop | 1 | 5.0 | Gallop | 3 |
| Gallop | 1 | 6.0 | Gallop | 3 |
| Gallop | 1 | 7.0 | Gallop | 3 |
| Gallop | No set time | 8.0 | Gallop | 3 |
| Recovery | 10 | 1.7 | Walk | 0 |

Adapted from Couroucé-Malblanc and Hodgson (9).

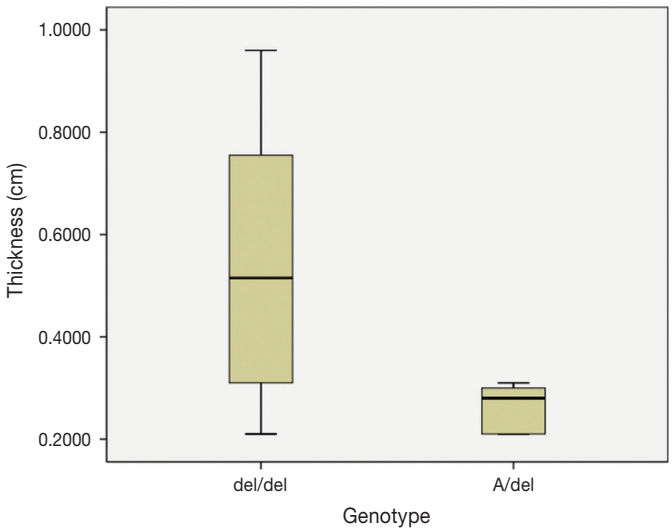


**Figure 1. Significant correlation ($P = 0.017$) between the mean thickness (± standard deviation) of the subcutaneous fat layer in the region of the gluteus medius, as measured by ultrasonography, and the *ACTN3* sequence variant c.1681–129delA in 17 Brazilian sport horses training for events in a tropical climate.**

Neither the c.1681–86G > A variant nor the c.1681–129delA variant of *ACTN3* intron 14–15 had previously been described, and these variants are distinct from the *ACTN3* haplotypes reported in horses by Mata et al (8) and Thomas et al (10).

The mean thickness (± SD) of the subcutaneous fat layer in the region of the gluteus medius was 0.464 ± 0.242 cm. Genotype and phenotype comparison revealed a significant correlation ($P = 0.017$) of the sequence variant c.1681–129delA with the thickness of the subcutaneous fat layer: the heterozygous A/del horses had a lower body fat thickness with less variability compared with the del/del homozygotes (Figure 1).

The horses maintained a speed of 8.0 m/s on the high-speed treadmill until becoming fatigued for 127 to 386 s, with a mean ± SD of 280.24 ± 74.52 s. Neither sequence variant was correlated with the time to fatigue.

An investigation in *ACTN3* mutant mice found a correlation between decreased weight gain and decreased size of the subcutaneous fat depot in individuals with predominantly slow type I muscle fibers (11). Thus, a switch in muscle metabolism could account for the findings in our study among the heterozygous horses with the variant c.1681–129delA. The phenotype of the horses training for events in a tropical climate may have influenced the selection of horses with these polymorphisms for our study. Brazil's hot and humid environment and the type of equestrian sport may have led to more adaptation of sports horses, as those with less subcutaneous fat would be more suited for an event in a tropical climate.

In conclusion, the c.1681–129delA mutation in the *ACTN3* intron 14–15, when homozygous, may have resulted in metabolic changes that led to increased deposition of body fat in Brazilian sport horses training for events in a tropical climate. This intronic single base-pair deletion should be considered for additional study in Brazilian sport horses and other equine breeds to evaluate its potential as a biomarker of phenotype and physical performance.

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