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A GENOME-WIDE ASSOCIATION STUDY FOR HEIGHT AT WITHERS IN RACING QUARTER HORSE

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SUMMARY

Height is a classic polygenic trait and an important conformation characteristic for horses. A genome-wide association study for height at withers was conducted in 120 racing Quarter Horses, which were genotyped using the Illumina EquineSNP50 Bead chip. Association analysis was performed with 40,787 SNPs (after quality control) using Qxpak5 software. The analysis revealed 8 chromosomal regions that harboured 23 SNPs associated ($P < 0.0001$) with height at withers. These regions were on chromosomes 3, 4, 5, 6, 7, 9, 17 and 30. A positional and functional candidate gene emerging from this study is *WISP1*, a gene previously related to bone development. Further studies are required to confirm these SNP associations and candidate genes in additional populations and other horse breeds.

INTRODUCTION

One of the highlights from the analysis of the horse genome project is its complete sequencing from a Thoroughbred animal (EquCab2.0) and, from this, the identification of 1,162,753 single-nucleotide polymorphisms (SNPs) in different breeds (Wade *et al*. 2009). As a result, genome-wide association studies (GWAS) based on SNPs and high density chips have been used to detect changes caused by genetic selection and to identify quantitative trait loci (QTLs). For example, GWAS have identified SNPs on *Equus caballus* autosomes (ECA) 18, within and proximal to the myostatin gene that are associated with racing performance in Thoroughbred horses (Hill *et al*. 2010). According to Signer-Hasler *et al*. (2012), less information is available on the genetics of polygenic quantitative traits in horses than in other species. Examples of polygenic traits are conformation traits including height at withers. Conformation traits are important criterion for selection and evaluation of a horse, particularly if a horse is a candidate for an athletic career (such as racing), long years of sound service or breeding (Thomas 2005). The objective of our study was to perform GWAS to identify chromosomal regions and positional candidate genes associated with height at withers in the racing Quarter Horse.

MATERIALS AND METHODS

Animals, Traits and Genotypes. Blood samples for DNA extraction were obtained from 120 racing Quarter Horses, born between 1985 and 2007 and registered at the Brazilian Association of Quarter Horse Breeders. Animals of this racing line, including 18 males and 102 females born to 48 stallions and 107 mares, were from five properties located in São Paulo state, Brazil. Height at withers was measure from the tallest point of the thoracic vertebrae to ground (Thomas 2005). This measure was performed by the same person with a tape measure and measuring stick, always

on the right side of the animal, with the horse standing with front and rear legs perpendicular to the ground. The DNA samples were genotyped with the Equine SNP50 BeadChip (Illumina, Inc., USA) using the HiScan system at the Faculty of Agricultural and Veterinary Sciences, Unesp, Jaboticabal, São Paulo, Brazil. This array contains 54,602 SNPs derived from the EquCab2.0 SNP Collection database, with a mean density of one SNP per 43.2 kb. Repeat samples were included for quality control and Genome Studio 2011.1 software (Illumina Inc., San Diego, CA) was used to call genotypes. SNP with call rates < 90%; cluster separation < 0.3; call frequency < 0.9; p-value < 1 x 10-3 for Hardy-Weinberg equilibrium and minor allele frequency < 0.05 were discarded. Quality control resulted in 40,787 autosomal SNPs for 120 animals.

Statistical Analysis. An association analysis was performed with the 40,787 SNPs for height at withers using Qxpak5 (Perez-Enciso and Misztal 2011) and fitting one SNP at a time. Qxpak5 relies on the well-known theory of mixed models, performing a likelihood ratio test with every SNP in turn, testing the model with the SNP versus the model without the SNP, against a chi-square distribution with 1 degree of freedom. Correction for multiple tests considered two metrics: false discovery rate (FDR) and Qvalue calculated with the package for R (Version 2.10). The percentage of the genetic variance accounted by the *i*-th SNP was estimated according to the following formula:

$$\%V_i = 100\left(\frac{2p_i q_i \hat{a}_i^2}{\sigma_g^2}\right)$$

where p_i and q_i are the allele frequencies for the *i*-th SNP estimated across the entire population, a_i is the estimated additive effect of the *i*-th SNP on the trait in question, and σ^2_g is the REML estimate of the (poly-)genetic variance for the trait.

RESULTS AND DISCUSSION

Descriptive statistics of the data are reported in Table 1. In accordance to previous reports (Signer-Hasler *et al*. 2012) moderate estimated heritability for height at withers was observed. Low phenotypic, genetic and residual variances were also observed.

Table 1. Summary statistics and heritability estimates for height at withers (meter) of the racing Quarter Horse

Parameter	Height at withers
N (animals)	120
Mean	1.55
Sample variance	0.002
Minimum	1.46
Maximum	1.72
σ^2_q	0.0008
h^2	0.58

We report the results of GWAS for height at withers using a mixed-model with fixed effect of sex and age at the time of trait measurement as a linear covariate. Twenty three significant SNPs were detected (P<0.0001) on ECA 3, 4, 5, 6, 7, 9, 17 and 30 (Figure 1 and Table 2). This *P*-value corresponds to FDR of 0.17 and q-values between 0.08 and 0.15, which are indicative of a possible true association, given the small sample size and the fact that each of these SNP accounted for an important proportion of the genetic variance (Table 2). On ECA 3, 4, 5, 7, 9 and 30 were found 2, 30, 19, 2, 2 and 1 genes located within the associated region, respectively. Among these results

some of the most interesting findings were on ECA 9. In ECA 6 and 17 no annotated genes were found within the associated region (1 Mb window around associated SNP).

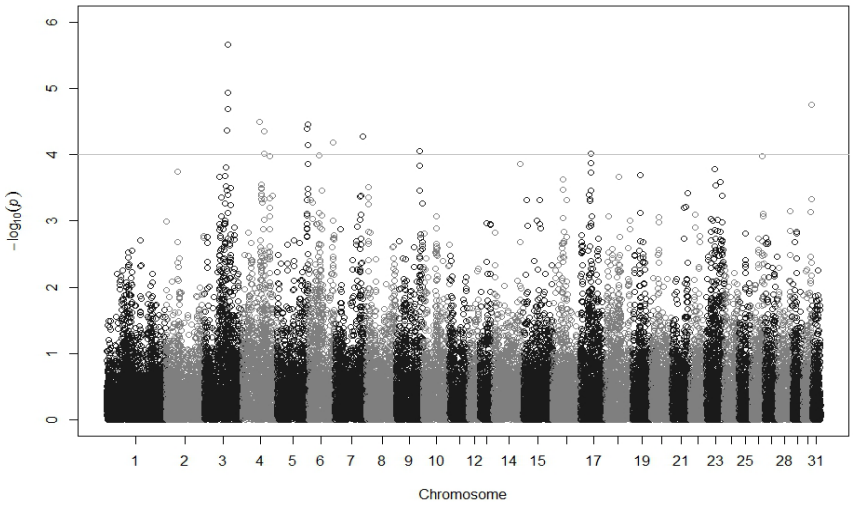

Figure 1. Manhattan plot of *P*-value for height at withers. The log inverse *P-values* estimated for each polymorphism are plotted in the y-axis. Chromosome number is plotted in the x-axis. Horizontal line indicates the threshold $P<0.0001$.

Signer-Hasler *et al*. (2012) identified eight SNPs within two QTL regions for height at withers on ECA 3 and ECA 9 in Franches-Montagnes horses. The two QTL regions are mapped near the *LCORL*/*NCAPG* (ECA 3) and *ZFAT* (ECA 9) genes. Tetens *et al*. (2013) have also identified significant association signal on the distal end of ECA 3 for height at withers in German Warmblood horses explaining ~18% of the phenotypic variance. In our study associations on ECA 3 and 9 were also reported but in different regions (implicating other genes: *LPHN3*, *TECRL* on ECA 3 and *WISP1*, *NDRG1* on ECA 9). Of these genes, *WISP1* is the one with a reported functional connection to growth and development playing an important regulatory role during bone development and fracture repair (French *et al*. 2004). This gene encodes a member of the *WNT1* inducible signaling pathway (WISP) protein subfamily, which belongs to the connective tissue growth factor (CTGF) family. *WNT1* is a member of a family of cysteine-rich, glycosylated signaling proteins that mediate diverse developmental processes (NCBI, 2013).

The percentage of the genetic variance explained by each SNP ranged 20.01% – 36.75% (Table 2), which was higher than normally encountered in GWAS (Tetens *et al*. 2013). These high values may be due to the small number of animals used in the study. Also, it is important to notice that these high percentages are in relation to a low genetic variance and that they were estimated in the same population used for the discovery of the SNP association. Despite these limitations, our results are similar to those of Makvandi-Nejad *et al*. (2012) that identified four loci on chromosomes 3, 6, 9 and 11, which together explained 83% of size variance in 48 horses from 16 breeds. According to Signer-Hasler *et al*. (2012), the genetic architecture of the digressed estimated breeding values (dEBV) for height at withers is characterized by a few genes with major effects and a large number of genes with small effects. Therefore, results reported here and elsewhere seem consistent.

Table 2. Effect, *P*-values and proportion of the variance explained for SNPs associated (*P*<0.0001) with height at withers of the racing Quarter Horse

SNP Name	ECA	Position	MAF	Effect	*P*-value	%Variance
BIEC2-789895	3	69,347,313	0.33	-0.021	4.29003E-05	24.19
BIEC2-789896	3	69,347,393	0.33	-0.021	4.29003E-05	24.19
BIEC2-789900	3	69,351,329	0.33	0.021	4.29003E-05	24.19
BIEC2-792718	3	73,581,276	0.38	-0.024	2.19999E-06	34.04
BIEC2-792768	3	73,797,941	0.34	0.022	2.03002E-05	27.05
BIEC2-792776	3	73,811,436	0.34	-0.022	2.03002E-05	27.05
BIEC2-792783	3	73,813,893	0.34	0.022	2.03002E-05	27.05
BIEC2-792814	3	73,881,123	0.38	-0.023	1.16001E-05	31.23
BIEC2-792839	3	74,052,752	0.34	0.022	2.03002E-05	27.05
BIEC2-866887	4	54,805,325	0.38	-0.02	3.18002E-05	23.44
BIEC2-869000	4	66,635,563	0.36	0.022	4.49997E-05	27.82
BIEC2-869084	4	67,096,727	0.35	-0.021	9.66006E-05	24.94
BIEC2-929949	5	93,714,559	0.48	-0.02	4.06004E-05	24.94
BIEC2-931221	5	95,656,828	0.18	-0.027	7.05992E-05	26.31
BIEC2-931466	5	95,894,134	0.40	0.021	3.51002E-05	26.55
BIEC2-931509	5	95,951,807	0.18	-0.027	7.05992E-05	26.31
BIEC2-931513	5	95,955,507	0.18	-0.027	7.05992E-05	26.31
BIEC2-931518	5	95,959,168	0.18	-0.027	7.05992E-05	26.31
BIEC2-1186793	6	76,390,403	0.45	0.018	6.58006E-05	20.01
BIEC2-1011792	7	87,083,836	0.18	0.028	5.33998E-05	28.48
BIEC2-1105149	9	73,886,834	0.42	0.02	8.71004E-05	24.37
BIEC2-375392	17	33,111,862	0.49	-0.018	9.5801E-05	20.24
BIEC2-828161	30	26,347,707	0.25	-0.028	1.75001E-05	36.75

CONCLUSION

Genomic regions on ECA 3, 4, 5, 6, 7, 9, 17 and 30 were associated with height at withers in the racing Quarter Horse. A total of 56 genes mapped to these regions and thus emerge as positional candidates for height at withers. However, most of these genes have no obvious function related to height. A positional and functional candidate gene from this study is *WISP1*, a gene related to bone development. Further studies are required to confirm these SNP associations and candidate genes in other populations and breeds.

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