



## A SNP in *ASAP1* gene is associated with meat quality and production traits in Nelore breed

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### ABSTRACT

The *ASAP1* gene is located in a QTL region for meat production traits and to access the role of the *ASAP1* gene, the association between a SNP in this gene and production traits in beef cattle was studied. For this, about 270 steers of reference families of Nelore breed were used. The investigation of marker effects on the traits was performed using a mixed model under the restricted maximum likelihood method. Novel association of a SNP in the *ASAP1* gene and shear force measured at 24 h post mortem ( $P \leq 0.0083$ ) was described in this population of Nelore cattle. This polymorphism accounted for 1.13% of the total additive variance and 17.51% of total phenotypic variance of the trait, suggesting that this marker could be used in marker assisted selection.

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The Brazilian cattle herd is composed of 80% pure and crossbred Zebu (*Bos indicus*) and among them, the Nelore Zebu breed has the greatest prominence, due to its levels of productive performance and adaptability to a tropical climate. Since Brazil has an important role in the livestock market as the first commercial bovine herd in the world, there is a special interest in improving carcass and meat quality.

Along with traditional selection, which has achieved significant results, marker assisted selection can help improve economically important traits earlier in the breeding cycle. Knowledge of genetic variation and the search for candidate genes or genomic segments that influence production traits in the population of interest are essential for the establishment of a molecular criteria for selection.

The gene *ASAP1* (*ArfGAP with SH3 domain, ankyrin repeat and PH domain 1*), also called *DDEF1*, gene ID: 327705; is a positional candidate gene since it is located on the bovine chromosome 14 where there are reports of QTLs for meat production traits including fat thickness, yield grade, and marbling (Casas et al., 2003). Some genes, like thyroglobulin (Barendse et al., 2001), *CHR* (Wibowo, Michal, & Jiang, 2007)

and *FABP4* (Michal, Zhang, Gaskins, & Jiang, 2006) have been indicated as candidates located in this QTL to influence these traits.

This study aimed to investigate the association between a SNP (NCBI\_ss181800428) in *ASAP1* candidate gene and backfat thickness (BFT), ribeye area (REA) and shear force (SF), in reference families of Nelore beef cattle.

To achieve this goal, 270 Nelore steers descending from 20 sires, which were chosen to represent the main genealogies marketed in Brazil, were investigated. However, for the analyses of SF at different maturation times, only data from 137 animals were available. The half-sib families were produced by artificial insemination in commercial Nelore dams. The animals were raised on three farms and at approximately 18 months of age they were allocated to two feedlots, where they were maintained for 90 to 130 days. The amount of feed offered (total mixture composed of corn silage and concentrate) was adjusted by the remaining feed, ensuring that the steers were fed *ad libitum*.

Twenty-four hours after slaughter, 2.5 cm thick steaks corresponding to a cross section of the longissimus dorsi muscle between the 12th and 13th ribs were collected. Steaks were identified, vacuum packed and used to measure BFT, REA, and SF. The BFT was measured with a ruler and the REA with a grid. The SF was obtained in the texture analyzer TA – XT2i coupled to a Warner–Bratzler blade with 1.016 mm thick. The analyses of SF were done at different maturation times: about 24 h after slaughter ( $t=0$ ), after seven days ( $t=7$ ) and 14 days ( $t=14$ ) of

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maturation at 2 °C in cold chamber manufactured at Macquay Heatcraft do Brasil Ltda, São José dos Campos, São Paulo, Brazil.

Straws of frozen semen obtained from Brazilian artificial insemination centers were used to extract DNA for bulls using a modification of the standard phenol–chloroform method (Sambrook, Fritsch, & Maniatis, 1990). Briefly, 250 µL of semen were rinsed with an equal volume of lysis buffer (1 mL) (10 mM Tris, 10 mM EDTA, 50 mM NaCl and 2% SDS) followed by a centrifugation at 16,000×g for 5 min and the supernatant was removed, leaving only 50 µL of buffer. Thereafter, 500 µL of lysis buffer were added, 40 mM DDT and 0.3 mg proteinase K. The cells were incubated overnight at 56 °C, while shaking. The protein precipitation was performed by adding an equal volume (500 µL) of phenol:chloroform:isoamyl alcohol (25:24:1 v/v). After homogenization the organic and aqueous phases were separated by centrifugation at 16,000×g for 15 min. The upper aqueous phase was transferred to a fresh tube. DNA precipitation was done by adding 600 µL of isopropanol 100% followed by a centrifugation at 16,000×g for 15 min. The pellet of DNA was rinsed with 1 ml of 70% ethanol and centrifuged at 16,000 g for 5 min. The ethanol was removed and the pellet was allowed to dry for 1 h. The DNA was dissolved in TE (10 mM Tris and 1 mM EDTA) with 10 µg/mL RNase, incubated at 37 °C for 1 h, quantified with a spectrophotometer, and stored at –20 °C.

For the steers, 5 ml of blood samples were used and DNA extractions were performed by a salting out method (Méo et al., 2009).

The SNP in intron 13 of the *ASAP1* gene was genotyped by an adapted ARMS methodology described by Veneroni et al. (2010).

To evaluate the influence of this DNA marker on BFT, REA and SF, a mixed model was used with fixed effects of contemporary groups (CG) and genotypes and the linear effect of age of the animal at measurement as a covariate, in addition to the random effect of the bull. For the analyses of SF, the pH measured in the meat at 24 h post slaughter was included as a covariate. The CG for BFT and REA included the effects of the month of birth, birth and feedlot places, resulting in 13 CG. One CG had less than four animals and was excluded from the analysis. For SF, the effect of slaughter batch was also included and 13 CG were considered. The mathematical model is described below. For BFT and REA the covariate pH was not included in the model.

$$Y_{ijkl} = \mu + GC_i + M_j + T_k + b_1(A_{ijkl-a}) + b_2(P_{ijkl-p}) + e_{ijkl}$$

Where:

$Y_{ijklm}$	observation of m-th individual, of age A son of k-th bull, of j-th genotype for the marker, belonging to the i-th contemporary group;
$\mu$	overall mean;
$GC_i$	fixed effect of the ith contemporary group;
$M_j$	fixed effect of jth genotype for the marker;
$T_k$	random effect associated with the kth animal's father, $N(0, \sigma^2)$ ;
$b_1$	regression coefficient associated with animal's age;
$A_{ijkl}$	animal age on the date of measure, a is the mean age of measure;
$b_2$	regression coefficient associated with sample pH;
$P_{ijkl}$	value for each observation of pH, p is the mean pH of the samples;
$e_{ijkl}$	random error associated with each observation $ijklm$ , assumed to be normally distributed with mean zero and variance $\sigma^2$ .

Analyses were performed by using PROC MIXED in the statistical analyses system (SAS) (SAS Institute Inc., 2000) and least-squares means were estimated for each genotype. The Bonferroni correction was applied to avoid false associations due to multiple tests.

Allele substitution effects were estimated by replacing the marker genotype effect with covariates corresponding to the number of copies of a given allele in the statistical model. As described by Schenkel et al. (2005), assuming the estimated allele frequencies for *ASAP1*, and using the estimated additive ( $a = 1/2$  homozygous genotype 1 –  $1/2$  homozygous genotype 2) and dominance deviation ( $d =$  heterozygous genotype –  $[1/2$  homozygous genotype 1 +  $1/2$  homozygous genotype 2]) effects for the alleles, the percentage of phenotypic variation explained by a marker was calculated using standard formula (Falconer & Mackay, 1996):  $\%V = (100 \times (2pq[a + d(q - p)]^2 + [2pqd] 2) / \sigma^2 p)$ , where %V is the percentage of phenotypic variation explained by the SNP, and  $\sigma^2 p$  is the phenotypic variance of the trait.

Results of the mixed model analyses of association among the marker and phenotypes under evaluation revealed a significant association of *ASAP1* polymorphism with SF ( $t = 0$ ) (Table 1).

The *ASAP1* gene is located in the bovine chromosome 14 which there are reports of QTLs for fat thickness, yield grade, and marbling (Casas et al., 2003).

A study conducted by King, Hu, Harris, et al. (1999) indicated that the *ASAP1* gene has an important role in the signal transduction process, in which a signal is passed across a cell to trigger a change in the activity or status of another cell, encoding a signal transduction protein involved in the differentiation of fibroblasts into adipocytes and possibly other cell types. Our work is the first to report an association between the *ASAP1* gene and SF ( $t = 0$ ). This polymorphism accounted for 1.13% of the total additive variance and 17.51% of total phenotypic variance of SF ( $t = 0$ ) in this population. We have to considerate that the SNP effect on trait variation could be biased by the small sample size analyzed, so including more animals in these analyses can help confirm the results obtained here. Although the only proven role of *ASAP1* gene in tissue differentiation is related to adipocytes, the involvement in other cell differentiation process is suggested in the literature (King et al., 1999). From our results it is possible to speculate that this gene could be involved in the differentiation of muscular fibers as well. Else it could be in linkage disequilibrium with the functional gene involved in this trait. The association of *ASAP1* with meat tenderness is supported by results obtained by Casas (2010, personal communication to Regitano, L.C.A.) who found an association of this gene with marbling, SF, and hot carcass weight in *Bos taurus* beef cattle. We have to considerate that the SNP effect on trait variation could be biased by the small sample size analyzed, so including more animals in these analyses can help to confirm the results obtained here. Although this gene is located in a QTL for backfat thickness and had been associated with this trait in other studies (Veneroni et al., 2010), association between this SNP in *ASAP1* gene and BFT was not confirmed in the Nelore population used in the present study. For REA a P-value near to the significance level ( $P \leq 0.01$ ) after Bonferroni correction was observed. From the estimated means for *ASAP1* genotypes (Table 1) it is shown that the heterozygotes had lower SF than either homozygote. The allele substitution effect of *ASAP1* marker was not significant ( $P \leq 0.05$ ). This result may have

**Table 1**

Summary of the results of the mixed model analyses of association among the traits evaluated and the marker, least-squares means for *ASAP1* genotype and standard errors for the traits by marker genotype.

<i>ASAP1</i>	Traits				
	BFT (mm)	REA (mm)	SF/t = 0 (kg)	SF/t = 7 (kg)	SF/t = 14 (kg)
N	270	270	137	137	137
P-value	0.660	0.011	0.008*	0.487	0.081
AA	6.59 ± 0.28 <sup>a</sup>	60.56 ± 0.87 <sup>a</sup>	8.47 ± 0.23 <sup>a</sup>	5.25 ± 0.34 <sup>a</sup>	3.70 ± 0.18 <sup>a</sup>
AG	6.77 ± 0.35 <sup>a</sup>	59.73 ± 1.06 <sup>a</sup>	7.80 ± 0.29 <sup>b</sup>	4.98 ± 0.31 <sup>a</sup>	3.38 ± 0.23 <sup>a</sup>
GG	7.29 ± 0.90 <sup>a</sup>	51.56 ± 3.03 <sup>a</sup>	10.8 ± 1.23 <sup>a</sup>	6.37 ± 1.5 <sup>a</sup>	5.53 ± 1.03 <sup>a</sup>

N = Number of animals measured.

Different superscripted letters within a column denote differences among means.

\* Significant association after Bonferroni correction.

**Table 2**  
Allelic and genotypic frequencies of *ASAP1* marker.

Marker	Frequency (%)				
	Allelic		Genotypic		
<i>ASAP1</i>	A	G	AA	AG	GG
	83	17	68.83	28.34	2.83

been influenced by the low frequency of animals homozygous GG (Table 2). Including more animals in future studies could provide better parameter estimation. Future studies with *ASAP1* expression could also clarify whether transcriptional differences would be related to phenotypic variation of this trait.

If the associations between this SNP in the *ASAP1* gene and SF, is proven in other populations of Nelore, this information could be used in marker assisted selection since these are highly valuable production traits in beef production.

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