



Association of the polymorphism *g.8514C>T* in the *osteopontin* gene (*SPP1*) with milk yield in the dairy cattle breed Girolando

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Source/description: The genomic DNA of 434 Girolando animals was amplified for the fourth intron of the *osteopontin* gene (*SPP1*) (HUGO nomenclature: *secreted phosphoprotein 1*) and then digested with restriction enzyme BsrI to detect the alleles of this SNP by PCR-RFLP. Three genotypes were identified in the population, TT, CT and CC (52.53%, 38.71% and 8.76%), respectively, and these were used for an association study with data on 305 day milk yield (M305) and predicted transmitting ability for milk yield (PTAM). Recorded milk data were from the Dairy Control Service of the Brazilian Association of Breeders Girolando managed by Embrapa Dairy Cattle. The predicted values of transmitting ability for milk (PTAM) for bulls and their daughters were taken from the national breed genetic evaluation programme in 2009 by Embrapa; these values are adjusted in relation to the genetic basis (7.59 kg), defined as the mean genetic 646 cows born in 2000.

DNA extraction and PCR conditions: Genomic DNA was extracted from blood and semen using the Blood & Tissue Dneasy kit (Qiagen). The polymorphism in the *SPP1* gene was investigated through sequencing across the SNP *g.8514C>T* located in intron 4 (GenBank GU143824.1) using primers described in the literature¹. The PCR products were subjected to restriction using the enzyme BsrI, which distinguishes alleles C and T of the SNP (Fig. 1).

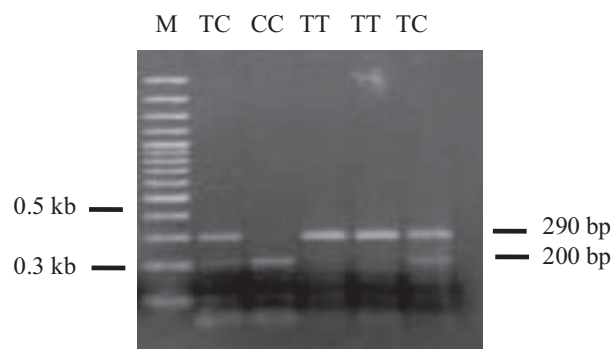


Figure 1 Agarose gel electrophoresis (1.5%) showing genotypes of the *SPP1* gene after digestion of the PCR fragment with Bsr I. The genotypes (TT, TC or CC) are given at the top of each lane. An expected 90-bp fragment in CT and CC genotypes was not seen on the gel. M, 1000–100 bp marker; PCR, undigested PCR fragment.

Table 1 Number of animals analysed for each trait (*n*), Holstein–Gir genetic composition, estimates of the effects of additive dominance, estimates of allelic substitution ($\alpha/2$) and *P*-value associated with the polymorphism *g.8514C/T* of the *osteopontin* gene (*SPP1*) in a population of Girolando cows and bulls.

Variables evaluated	<i>n</i>	Genetic composition	Substitution allelic ($\alpha/2$)	Additive effect	Dominance effect
M305	159	5/8	–	31.06 (0.8509)	366.21 (0.1225)
PTAM cows	159	5/8	12.29 (0.1973)	–	–
PTAM bulls	32	3/4 and 5/8	19.69 (0.5612)	–	–

Analysis: Recorded milk data of a small number of animals were available for association analysis. Thus, data from 191 animals were used in the association study. For the analysis of PTAM, data from 32 bulls and 159 primiparous daughters were used. The positive effects of an additional allele are necessarily additive, so the model used to analyse the effect of allelic substitution in PTAM data included: fixed effects of bull, the regression coefficient of number of alleles *C* (0, 1 or 2) at the *SPP1* gene locus, and the residual effect. For analysis of M305, the data of 159 daughters were used. The following model fixed effects were included in the model: bull, contemporary group (herd and season of birth of the cow), genetic composition and residual effects. The additive genetic effect of locus was estimated as half the difference between the two groups of homozygotes ($\hat{\sigma}_{CC} - \hat{\sigma}_{TT}/2$). The dominance effects were estimated as the difference between the group of heterozygotes and the average of two groups of homozygotes at that locus. The association was performed by linear regression analysis² (Appendix S1).

Association: No significant association was found between the alleles of the polymorphism and the characteristics evaluated in this study (Table 1), although the highest milk production was observed in animals with at least one copy of the T allele.

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References

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Supporting information

Additional supporting information may be found in the online version of this article.

2 Brief Note

Appendix S1 Statistical analysis.

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