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SHORT COMMUNICATION

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Bovine thyroglobulin gene polymorphisms and their association with sexual precocity in Guzerat bulls

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Contents

Puberty is a stage of sexual development determined by the interaction of environmental factors and genetic mechanisms. Among them, thyroid function plays a key role in sexual development and spermatogenic function and is under the control of several genes, including the well-described thyroglobulin gene (*TG*). Previous reports have shown genetic association between thyroid function and selected single nucleotide polymorphisms (SNPs) in taurine cattle. Therefore, the identification of genetic mechanisms involved in the regulation of this trait can assist with the selection for early pubertal bulls, thus improving genetic progress in livestock breeding. The aim of this study was to validate the association between *TG* SNPs and age at puberty in zebuine bulls. Three SNPs (rs110406764, rs109662686, rs109057985) were genotyped in 159 Guzerat animals using SEQUENOM technology. Results showed a significant association ($p < .05$) between the studied SNPs and puberty age, in agreement with our previous reports in a taurine breed. Interestingly, allele frequencies were different from those already reported, being GAT the most favourable allele for age at puberty in Guzerat (94.4 days lower). Overall, our findings corroborate previous reports and reinforce the importance of genetic influence in the regulation of sexual development and puberty through a thyroid pathway in zebuine cattle.

1 | INTRODUCTION

Puberty is an important target for genetic improvement in cattle as it allows an early use of the individuals as breeders, reducing the generation interval and increasing fertility and genetic progress (Fortes et al., 2012; Johnston et al., 2009). Therefore, early prediction of this trait would enable its inclusion in breeding schemes. Furthermore, identification of new genetic variations for puberty regulation could extend our knowledge of physiology and simultaneously assist with the selection of early pubertal bulls.

Guzerat is a millenary zebuine beef breed native from India extensively used in South American tropical breeding systems. Fertility rates are average in this breed, mainly due to later sexual development in comparison with European (*Bos taurus*) breeds, becoming an important constraint in beef production systems (Nogueira, 2004). Although puberty onset in Guzerat bulls ranges from 14 to 25 months of age, the high variability of this trait among individuals facilitates genetic improvement.

Sexual development in the Guzerat breed has been widely characterized phenotypically (Loaiza-Echeverri et al., 2013; Torres-Júnior & Henry, 2005). However, genetic association studies have not determined genetic markers which can help to predict the reproductive precocity of these animals.

In a previous report, we determined the association of three single nucleotide polymorphisms (SNPs) located in the thyroglobulin gene

(*TG*) with age at puberty in young Angus bulls based on sperm quality criteria (Fernández et al., 2014). This association was explained by the connection of thyroid hormones (TH) with nearly every biological endocrine system, but particularly with the hypothalamic-pituitary-gonadal axis, responsible for sexual development (Duarte-Guterman, Navarro-Martín, & Trudeau, 2014; Weber, Vigone, Stroppa, & Chiumello, 2003). Furthermore, a biological connection between metabolic status and sexual development mediated by androgenic regulation of TH synthesis has been shown. Therefore, we aimed to validate the genetic association previously described in a taurine breed in a *Bos taurus indicus* breed (Guzerat) for future use in ongoing genomic breeding programs of several South American countries. Additionally, we aimed to determine the stability of genetic association among major cattle types in order to contribute to the genetic characterization of puberty onset mechanisms.

2 | MATERIALS AND METHODS

This study was conducted in three farms located in Minas Gerais, Brazil. Data from 159 Guzerat bulls born between 2001 and 2010 (sired by 48 different bulls) were used. After weaning, all calves were raised under grazing conditions. Scrotal circumference (SC) and body weight were obtained from 305 to 1,030 day-old bulls at approximately 3-month intervals. Semen was collected by electroejaculation every 3 months approximately after SC reached 20 cm. Puberty onset was estimated under two criteria: (i) SC of 26 cm (testicular puberty, TP; Fortes et al., 2012); (ii) presence of the first motile spermatozoa in the ejaculate (motility puberty, MP; Torres-Júnior & Henry, 2005). If motile spermatozoa were observed 3 months after an unsuccessful attempt, puberty onset was set as half the time between the unsuccessful and successful semen collections. In this way, detection of a low number of sperm cells with low motility, presence of proximal droplet and a high index of abnormal cells in the ejaculate determined puberty onset on the day of the measurement. Contrarily, and in most cases, detection of a high concentration of sperm cells, a high percentage of motility and low incidence of proximal droplet showed that those animals had passed through the beginning of puberty at some moment between the present and the previous sampling; so, average values between both measurements were taken. Finally, in a few animals with extreme values, age at puberty was moved from the average towards the first quarter of the interval if motility was too high and no proximal droplet was observed.

Three genetic markers (rs110406764, rs109662686, rs109057985) located in the 3′ flanking region of *TG* (Hou et al., 2011) were genotyped in 159 Guzerat bulls using SEQUENOM platform (GeneSeek Inc. Genotyping Services, Lincoln, NE, USA). To evaluate linkage disequilibrium (LD) of the studied SNPs, haplotypes were constructed for each individual using Phase algorithm (Li & Stephens, 2003) and visualized using Haploview (Barrett, Fry, Maller, & Daly, 2005).

The association between genetic markers and the estimated age at puberty was analysed using PROC MIXED (SAS 9.0 software, SAS Inst. Inc., 2002, Cary, NC, USA) with the linear mixed model, as follows:

$$Y_{ijkl} = \mu + S_i + G_j + B_k + O_l + e_{ijkl},$$

where Y_{ijkl} is the phenotypic observation of the I bull, μ is the overall mean, S_i is the fixed effect of i_{th} year, G_j is the fixed effect of j_{th} genotype, B_k is the fixed effect of k_{th} herd, O_l is the random effect of l_{th} sire and e_{ijkl} is the random error. The percentage of the genetic variance accounted by the j-th genotype was computed according to Falconer and Mackay (1996).

3 | RESULTS AND DISCUSSION

Linkage disequilibrium (LD) analysis indicated that SNPs were linked; in the case of rs109662686 and rs109057985, they were completely linked ($R^2 = 1$). Three different haplotypes were found, AGG, GGG and GAT, with frequencies of 0.877, 0.013 and 0.11, respectively. AGG/AGG was the most frequently detected genotype (0.786), followed by AGG/GAT (0.188) and GAT/GGG (0.019). AGG/GGG was detected in only one bull (0.007) and therefore excluded from the association analysis.

Single nucleotide polymorphisms (SNPs) and haplotypes were tested for association with phenotypic data corresponding to the two estimated puberty ages mentioned above, showing no association with TP. However, a significant association ($p = .0039$) was detected between haplotype markers and MP, presenting individual p-values of .0042 for rs109662686 and rs109057985, and .0041 for rs110406764. Such difference could be due to several factors, including the hypothesis that TG would have a greater participation in the pathways and events that lead to an increase in sperm quality than in those specifically involved in testicular development.

Age at puberty was significantly lower in AGG/GAT (595.48 ± 27 days) than in AGG/AGG (691.89 ± 16 days), resulting in a difference of 96.4 days ($p = .0027$). On the other hand, GAT/GGG (679.87 ± 82 days) was found in three animals only, and was therefore not statistically significant for the analysis. The associated haplotypes explained 0.87% of the genetic variance of age at puberty at first motile spermatozoa.

The present results are in agreement with our previous findings that GAT/GAT induced a puberty forwardness of 58 days compared with AGG/AGG, and 10 days compared with the heterozygous (GAT/AGG). However, the frequency of AGG/AGG, the most common haplotype detected in this study, was extremely low (0.03) in taurine breeds (Fernández et al., 2014). Our results demonstrated that the GAT haplotype significantly lowered age at puberty in the Guzerat breed ($p = .0027$).

A previous study performed in Angus breed (Fernández et al., 2014) showed that TGT and CAG haplotypes were strongly linked to puberty onset. Our present results demonstrate that GAT and AGG haplotypes behaved similarly in Guzerat. Thus, the results observed between haplotypes (TGT with GAT and CAG with AGG) could be used as analogues as two SNPs were the same (rs110406764 and rs109057985) and the third pair (rs109662686 and rs378215592) was in complete genetic linkage.

After comparison, AGG frequency was 0.87 in Guzerat and 0.19 (detected as CAG) in Angus. Conversely, GAT frequency was 0.11 in

Guzerat and 0.81 (detected as TGT) in Angus. The detected positive association for age at puberty explained 0.87% of the trait variance.

The joint analysis of data showed that the results obtained in two different breeds fitted considerably. Interestingly, the haplotypes which explained the lower age at puberty were detected in very high frequencies in Angus, a breed characterized by an early puberty onset (Lunstra & Cundiff, 2003). On the contrary, Guzerat, characterized by a late puberty onset, showed an increased frequency of the haplotypes associated with a delay in this trait. Thus, these results validate the implication of *TG* and its polymorphisms at some stage during the way to the onset of puberty. Consequently, *TG* can be considered a promising candidate gene to partially explain puberty development in cattle.

In conclusion, our results validate that the previously reported association between *TG* polymorphisms and age at puberty in *Taurus* cattle maintained in a zebuine breed. Further research, probably including the study of a composite *taurus* x *indicus* breed could help determine a higher number of haplotype variants and thus improve the understanding of puberty onset mechanisms in cattle, allowing to select more productive individuals.

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AUTHOR CONTRIBUTIONS

JPL, GG and MEF conceptualized and supervised the whole study. AMLE, MH, DAAO and DCC collected the phenotypic data and estimated puberty indexes. MD performed SNP genotyping. JPL, MEF and GG analysed the data. JPL, MEF, GG and SDP drafted and revised the manuscript. All authors contributed to this work and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICS APPROVAL

The study was approved by the Ethical Committee for Use of Experimental Animals of the Federal University of Minas Gerais, Brazil (Project Title: Comparação de cinco modelos não lineares para descrever o crescimento do perímetro escrotal em touros da raça Guzerá. CETEA Protocol no. 115/09 approval—08 2009).

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