RAPID COMMUNICATION: Multi-breed validation study unraveled genomic regions associated with puberty traits segregating across tropically adapted breeds¹

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ABSTRACT: An efficient strategy to improve QTL detection power is performing across-breed validation studies. Variants segregating across breeds are expected to be in high linkage disequilibrium (LD) with causal mutations affecting economically important traits. The aim of this study was to validate, in a Tropical Composite cattle (TC) population, QTL associations identified for sexual precocity traits in a Nellore and Brahman meta-analysis genome-wide association study. In total, 2,816 TC, 8,001 Nellore, and 2,210 Brahman animals were available for the analysis. For that, genomic regions significantly associated with puberty traits in the meta-analysis study were validated for the following sexual precocity traits in TC: age at first *corpus luteum* (AGECL), first postpartum anestrus interval (PPAI), and scrotal circumference at 18 months of age (SC). We considered validated QTL those underpinned by significant markers from the Nellore and Brahman meta-analysis ($P \leq 10^{-4}$) that were also significant for a TC trait, i.e., presenting a P -value of $\leq 10^{-3}$ for AGECL, PPAI, or SC. We also considered as validated QTL those regions where significant markers in the reference

population were at ± 250 kb from significant markers in the validation population. Using this criteria, 49 SNP were validated for AGECL, 4 for PPAI, and 14 for SC, from which 5 were in common with AGECL, totaling 62 validated SNP for these traits and 30 candidate genes surrounding them. Considering just candidate genes closest to the top SNP of each chromosome, for AGECL 8 candidate genes were identified: *COL8A1*, *PENK*, ENSBTAG00000047425, *BPNT1*, *ADAMTS17*, *CCHCR1*, *SUFU*, and ENSBTAG00000046374. For PPAI, 3 genes emerged as candidates (*PCBP3*, *KCNK10*, and *MRPS5*), and for SC 8 candidate genes were identified (*SNORA70*, *TRAC*, *ASS1*, *BPNT1*, *LRRK1*, *PKHD1*, *PTPRM*, and ENSBTAG00000045690). Several candidate regions presented here were previously associated with puberty traits in cattle. The majority of emerging candidate genes are related to biological processes involved in reproductive events, such as maintenance of gestation, and some are known to be expressed in reproductive tissues. Our results suggested that some QTL controlling early puberty seem to be segregating across cattle breeds adapted to tropical conditions.

Key words: across-breed validation, *Bos indicus*, beef cattle, GWAS, sexual precocity, tropical cattle

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INTRODUCTION

The age at which cattle reach puberty is a determining factor for the profitability of the beef industry. Selection for early puberty may allow producing more calves in less time, reduce generation interval, promote faster economic return, and higher genetic gains (Zhang et al., 2014). Although some breeding programs select *Bos indicus* breeds for sexual precocity, they are commonly reported as later to achieve puberty compared with *Bos taurus* breeds (Nogueira, 2004). The identification of genes and/or chromosome regions significantly associated with puberty traits could contribute to a better understanding of the genetic mechanisms underlying these traits, which may help to develop more efficient strategies and tools to attain genetic improvement of *B. indicus* breeds.

Bos indicus is the predominant cattle subspecies in Brazil, corresponding to approximately 80% of the total population, with Nellore being the most prevalent breed. In the Northern region of Australia, *B. indicus* cattle are also predominant, with Brahman and Tropical Composite (TC) being the most prevalent breeds.

Genome-wide association studies (GWAS) were performed in livestock to investigate the genetic background of different traits, including reproductive traits (Fortes et al., 2011; Irano et al., 2016). Multi-breed GWAS may improve the power of mapping QTL compared with within-breed GWAS because the linkage disequilibrium (LD) is expected to be conserved in short distances across breeds (Raven et al., 2014). Furthermore, combining information from different breeds increases sample size and detection power (van den Berg et al., 2016). Another strategy to improve the precision of QTL detection is performing cross-validation studies, since mutations segregating across breeds are very likely to be in high LD with the causal variant (Saatchi et al., 2014).

The aim of this study was to validate associations for sexual precocity traits from a meta-analysis study that used Nellore and Brahman as the reference population, by using Tropical Composite cattle as the validation population.

MATERIALS AND METHODS

All managements and procedures involving production, maintenance, and use of Nellore animals were certified and approved by the National Council of Animal Experimentation Control (CONCEA, 2008) and Use Committee at University of Sao Paulo, Jaboticabal Campus (18.340/16). Regarding

Brahman and TC animals, Animal care and Use committee approval was not required because the data are from existing databases described in the following section.

Phenotypes and Genotypes

Nellore information was available from the Alliance Nellore dataset (Brazil) and Brahman and TC data were obtained by the Cooperative Research Centre for Beef Genetic Technologies (Beef CRC, Australia). Nellore and Brahman phenotypes and genotypes used in the meta-analysis study are described in Melo et al. (2018). The Beef CRC datasets were previously reported in breed-specific GWAS (Hawken et al., 2012; Fortes et al., 2012, 2013).

Nellore traits used in the meta-analysis study were age at first calving (AFC), early pregnancy (EP), and scrotal circumference measured at about 18 months of age (SC_N). In total, 1,796, 1,849, and 4,248 animals with phenotypic and genotypic information were available for AFC, EP and SC_N , respectively. Brahman traits considered in the meta-analysis were age at first *corpus luteum* (AGECL), first postpartum anestrus interval (PPAI), both measured in days, and scrotal circumference at about 18 months of age (SC_B), measured in centimeters. The total number of Brahman animals with phenotypic and genotypic information available for these traits was 1,007 for AGECL, 629 for PPAI, and 1,203 for SC_B .

The Illumina High-Density (HD) Bovine SNP and GeneSeek Genomic Profiler Indicus HD – GGP75Ki (Neogen Corporation, Lincoln, NE) panels were used to genotype Nellore animals. Imputation from GGP75Ki to HD panel was performed using the FImpute v2.2 software (Sargolzaei et al., 2014). The quality control applied to Nellore genotypes were: removing animals with call rate < 0.90 and SNP with minor allele frequency (MAF) < 0.01, call rate < 0.95, GC score < 0.15, Hardy–Weinberg equilibrium test ($P < 10^{-5}$), SNP in nonautosomal regions, and unmapped SNP. After quality control, the final data contained 2,923 females genotyped for 412,993 SNP and 5,078 males genotyped for 477,317 SNP.

Experimental design, origin, phenotypes, and breed composition of TC animals were described by Barwick et al. (2009), Johnston et al. (2009), and Burns et al. (2013). TC traits used in the validation study were the same described for Brahman: AGECL, PPAI, and SC. In total, 1,097, 1,097, and 1,719 TC animals with phenotype and genotype were evaluated for AGECL, PPAI, and SC, respectively.

Illumina BovineSNP50 genotypes for Brahman and TC animals were imputed to HD with the Beagle software (Browning and Browning, 2009), using sires and additional representative animals of the Beef CRC population as the reference population for imputation (Fortes et al., 2013). For both Brahman and TC, quality control excluded animals with call rates lower than 98%, SNP with call rates lower than 85% and SNPs with MAF <0.02. Quality control differed between breeds because the genotypes were previously imputed and cleaned for all populations before performing the meta-analysis. After quality control for Brahman, a total of 625,041 SNP remained for females and 612,992 SNP remained for males to the analysis. For TC, those numbers were 683,671 SNP for females and 682,757 SNP for males.

Meta-analyses GWAS and Validation

The meta-analysis was performed using a multi-trait approach (Bolormaa et al., 2014). In summary, this method consists of a χ^2 statistic test with n degrees of freedom, where n is the number of traits included in the analysis ($n = 6$). The total number of SNP included in this meta-analysis was 387,971 SNP, which were in common among Nellore and Brahman panels. Details about this procedure and the GWAS for Nellore and Brahman were described in Melo et al. (2018).

For the validation population, SNP effects were estimated in turn by using the EMMAX method (Kang et al., 2010), under the following model:

$$y = X\beta + sa + Zu + \varepsilon,$$

where y is a vector with the phenotypes, X is an incidence matrix relating fixed effects (contemporary group for all traits and age of the young bull at the measurement for SC) in β with the phenotypes in y , s is the vector with the genotypes coded as 0, 1, or 2 according to the number of B allele copies, a is the vector with the SNP allelic substitution effects, Z is the incidence matrix of polygenic random effects of the animals in u and ε is the vector of residuals. u and ε followed a normal distribution with $u \sim N(0, G\sigma_a^2)$ and $\varepsilon \sim N(0, I\sigma_e^2)$, respectively, where G is the genomic relationship matrix for all individuals and SNP (except for the SNP considered in a), calculated by the first method proposed by VanRaden (2008), σ_a^2 and σ_e^2 are the additive genetic and residual variance, respectively, and I is an identity matrix. SNP effect estimates were obtained with the SNP and Variation Suite v8.3.0 software (Golden Helix, Inc., Bozeman, MT).

We considered validated those SNP that were significant for the meta-analysis using Nellore and Brahman ($P \leq 10^{-4}$) and that were also significant for TC traits ($P \leq 10^{-3}$). Additionally, if significant SNP in the reference population were located at ± 250 kb of distance from significant SNP in the validation population, we considered this region a validated QTL.

RESULTS AND DISCUSSION

A total of 296 significant SNP ($P \leq 1 \times 10^{-4}$) were identified in the meta-analysis of Nellore and Brahman populations. The number of significant SNP ($P \leq 1 \times 10^{-3}$) for TC was: 874 SNP for AGECL, 842 SNP for PPAI, and 918 SNP for SC. A total of 49, 4, and 14 SNP were validated for AGECL, PPAI, and SC, respectively. The top SNP of each chromosome for AGECL, PPAI, and SC are presented in Table 1.

QTL Around Validated SNP

Validated SNP were distributed over chromosomes 1, 14, 15, 16, 17, 21, 23, 26, and 29 for AGECL, and most of them were distributed over chromosome 14. For PPAI, validated SNP were distributed over chromosomes 1, 10, and 11, and, for SC, validated SNP were located on chromosomes 8, 10, 11, 16, 21, 23, 24, and 27, where chromosomes 10 and 21 harbored the most of these validated SNP (Table 1).

Some of the validated regions surround the top SNP of each chromosome presented in Table 1 were located in QTL regions previously reported for reproductive traits. For example, McClure et al. (2010) found significant markers on BTA10 at 100 Mb, on BTA27 at 41 Mb, and on BTA29 at 9 Mb associated with SC in Angus, these regions were close to validated SNP here for PPAI, SC, and AGECL, respectively. A SNP in the gene *NFKBIL1* located on BTA23 at 27.5 Mb was associated with reproductive traits in Holstein (Cochran et al., 2013), and was a validated region for AGECL. Höglund et al. (2014) also validated SNP for the length of the interval from calving to first insemination in cattle in this region (≈ 28 Mb). Furthermore, these authors found on BTA11 at 2.1 Mb an SNP associated with 56-day nonreturn rate of cows, which was a region validated for PPAI. The region of BTA14 at 25 Mb validated here for AGECL was previously reported to be associated with some reproductive traits, as in Liu et al. (2017) who performed a meta-analysis for female fertility traits in cattle,

Table 1. Number of validated SNP ($P < 1 \times 10^{-3}$) per *Bos taurus* autosome (BTA), the most significant SNP in each autosome (top SNP), its position, and its distance from the closest gene (± 250 kb) in base pairs for age at first *corpus luteum* (AGECL), postpartum anestrus interval (PPAI), and scrotal circumference (SC).

BTA ¹	Number of SNP	Top SNP	Position, bp ¹	Gene symbol	Distance, bp
AGECL					
1	1	BovineHD0100012377	43,458,869	<i>COL8A1</i>	83,067
14	24	BovineHD1400007314	25,241,366	<i>PENK</i>	18,375
15	1	BovineHD1500002332	9,064,376	<i>Uncharacterized Protein</i> (<i>ENSBTAG00000047425</i>)	141,535
16	1	BovineHD1600006716	24,282,102	<i>BPNT1</i>	0
21	17	BovineHD2100001408	6,839,238	<i>ADAMTS17</i>	0
23	2	BovineHD2300007697	27,782,568	<i>CCHCR1</i>	0
26	2	BovineHD2600006035	23,405,679	<i>SUFU</i>	47,301
29	1	BovineHD2900002706	9,174,027	<i>Uncharacterized Protein</i> (<i>ENSBTAG00000046374</i>)	1,952
PPAI					
1	1	BovineHD0100042609	147,345,274	<i>PCBP3</i>	0
10	2	BovineHD1000029240	100,990,620	<i>KCNK10</i>	0
11	1	ARS-BFGL-NGS-68030	1,901,787	<i>MRPS5</i>	0
SC					
8	1	BovineHD0800024244	81,614,440	<i>SNORA70</i>	50,413
10	4	BovineHD1000007157	22,121,623	<i>TRAC</i>	0
11	1	BovineHD1100029283	100,818,657	<i>ASS1</i>	0
16	1	BovineHD1600006716	24,282,102	<i>BPNT1</i>	0
21	4	BovineHD2100001045	5,655,578	<i>LRRK1</i>	0
23	1	BovineHD2300006383	23,921,232	<i>PKHD1</i>	0
24	1	BovineHD2400011464	41,304,824	<i>PTPRM</i>	0
27	1	ARS-BFGL-NGS-118334	41,297,038	<i>Uncharacterized Protein</i> (<i>ENSBTAG00000045690</i>)	240,958

¹SNP were mapped to the UMD3.1 Bovine Assembly.

Mota et al. (2017) who found a significant 1 Mb window for AFC in Nellore in this region (≈ 24.6 Mb), and Oliveira Júnior et al. (2017) who found important genomic regions for heifer pregnancy in this region and for antral follicles on BTA15 from 8 to 10 Mb in Nellore heifers, another region that was validated for AGECL.

It was observed that five SNP validated for AGECL were also validated for SC: “BovineHD2100001045” (BTA21 at 5 Mb), “BovineHD2100002586” (BTA21 at 10 Mb), “BovineHD2100002600” (BTA21 at 10 Mb), “BovineHD4100014975” (BTA21 at 10 Mb), and “BovineHD2300006383” (BTA23 at 23 Mb). Due to this, AGECL and SC presented three candidate genes in common, *LRRK1*, *NR2F2*, and *PKHD1*.

Genes Surrounding the Candidate Regions

There were a total of 30 candidate genes, i.e., the closest genes surrounding the validated SNP (Supplementary Table 1). However, for discussion only the candidate genes around the top SNP per chromosome will be considered (Table 1). The majority of the candidate genes identified here

(Table 1) were either associated with reproductive events or was expressed in reproductive tissues of mammals.

Candidate genes for AGECL were generally expressed in reproductive tissues. For example, *PENK*, located on BTA14 at 25 Mb, was expressed in bovine endometrium (Bauersachs et al., 2005). *BPNT1*, on BTA 16 at 24 Mb, presented differentially expressed protein levels in equine uterus between estrus and luteal phase (Soleilhavoup et al., 2015). *ADAMTS17* (BTA21 at 6 Mb) was up-regulated in the endometrium of pregnant pigs vs. nonpregnant pigs (Kim et al., 2012). *CCHCR1* (BTA23 at 27 Mb) was differentially expressed in bovine endometrium (Waters et al., 2014). *SUFU* (BTA26 at 23 Mb) was expressed in mouse testis and associated with spermatogenesis (Szczepny et al., 2005).

PCBP3 (BTA1 at 147 Mb), *KCNK10* (BTA10 at 100 Mb), and *MRPS5* (BTA11 at 1 Mb) emerged as candidate genes for PPAI. *PCBP3* protein was expressed in spermatids of rats during spermiogenesis (Chapman et al., 2013). *KCNK10* was associated with semen volume in bulls (Hering et al., 2014) and was expressed at the membrane of oocytes

and blastocysts of cows (Hur et al., 2009). *MRPS5* was expressed in primate primordial oocyte cells (Arraztoa et al., 2005).

Candidate genes for SC were: *SNORA70* (BTA8 at 81 Mb), *ASS1* (BTA11 at 100 Mb), *LRRK1* (BTA21 at 5 Mb), *PKHD1* (BTA23 at 23 Mb), and *PTRM* (BTA24 at 41 Mb). *SNORA70* was detected in genomic regions under positive signature selection for reproductive traits in cattle (Pitt et al., 2019). *ASS1* was associated with abortion in humans (Pangalos et al., 2016). *LRRK1* was associated with reproductive traits in pigs (Suwannasing et al., 2018) and with pregnancy outcome after fixed-time AI in cattle (Porto-Neto et al., 2015). *PKHD1* was associated with embryo development and survival in human (Gigarel et al., 2008). *PTPRM* was expressed in rat spermatogonia cells (Ryser et al., 2011).

ENSBTAG00000047425, *SUFU* and ENSBTAG00000046374 were candidates for the meta-analysis study using Nellore and Brahman reproductive traits (Melo et al., 2018). Also, *PENK*, another candidate gene for AGECL (Supplementary Table 1), was in a peak region of BTA14 of this meta-analysis study.

In this study, genomic regions previously associated with sexual precocity traits in a meta-analysis study using Brahman and Nellore were validated in Tropical Composite. Furthermore, genes associated with reproduction in different mammal species emerged as candidates. Thus, these regions are likely segregating across Brahman, Nellore, and Tropical Composite, and presenting pleiotropic effects for female and male sexual precocity.

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