Genome-wide association study for male and female reproductive performance indicator traits in Nellore cattle

J.M. Dementshuk,[1] G.A. Oliveira[2], B.C. Perez[3], B.F. Santana[5], J.P. Eler[4], J.B.S. Ferraz[4]

[1] Universidade Federal do Rio Grande do Sul, Departamento de Zootecnia, Porto Alegre, RS, Brasil.
[2] CRVLagoa, Sertãozinho, SP, Brazil.
[3] Universidade de São Paulo, Faculdade de Zootecnia e Engenharia de Alimentos, Pirassununga, SP, Brasil.
[4] Grupo de Melhoramento Animal e Biotecnologia (GMAB), Departamento de Medicina Veterinária, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP, Brazil.

Summary

Reproductive performance traits have a great economic influence on beef production. The scrotal circumference (SC) is often used as an inexpensive indicator of fertility and puberty but can be only applied to males. The probability of pregnancy at 14 months (PP14) is a precocity indicator trait and an alternative for direct measurements in females. The goals of the current study were to identify and characterize genomic regions explaining PP14 and SC. The EBVs of Nellore cattle were deregressed and treated as pseudo phenotypes in the genome-wide association analysis. Genomic prediction analysis was performed by genomic best linear unbiased (GBLUP) method, under a Bayesian approach. The top 10 windows with the higher percentage of genetic variance for SC were located in chromosomes 1, 7, 10, 11, 14, 16 and 22 and for PP14 in the chromosomes 5, 6, 10, 12, 17, 20 and 23. There were no overlapping regions in the top 10 windows for both PP14 and SC, indicating that both traits should be independently considered as selection criteria.

Keywords: reproductive traits, puberty, genomic selection.

Introduction

Reproductive performance traits have high economic impact on beef production. Generally, zebu cattle breeds are less precocious than taurine breeds, making selection for precocity relevant. For females, reproductive traits are often difficult and expensive to measure. The scrotal circumference (SC), an inexpensive measurement performed in males, is often used as an indirect indicator of fertility and puberty in females, being commonly measured in bovine animal breeding programs in Brazil. The probability of pregnancy at 14 months (PP14) is a precocity indicator trait and an alternative for direct measurements in the female for being easily obtained. As the SC can unsatisfactorily improve the ability of females to conceive when exposed at 14 months old (Van Melis *et al.*, 2010), knowing and characterizing genomic regions associated with PP14 and SC in Nelore cattle breed may help understanding the gene distribution that affects both traits and to define selection criteria that could support obtaining more precocious animals. The goal of the current study was to identify genomic regions explaining PP14 and SC.

Material and Methods

Phenotypic and genotypic data

Estimated breeding values (EBVs) for probability of pregnancy at 14 months (PP14) and scrotal circumference (SC) were obtained from routine genetic evaluations (CFM, 2017), comprising 27,546 (PP14) and 96,612 (SC) observations. Phenotypes used for genetic evaluations were collected after exposing heifers (around 14 months old) to a bull for a period of 120 days for PP14, while phenotypes for SC were collected from males around 18 months old. The EBVs were deregressed by the method described by Garrick *et al.* (2009) and treated as pseudo phenotypes in the genome-wide association analyzes. The accuracy threshold for the deregressed EBVs considered for the analyzes was 0.25.

A total of 1,296 and 1,883 individuals had genotype data for 777,962 SNPs (Ilumina® BovineHD BeadChip assay) for PP14 and SC, respectively. From those, 1,003 individuals had (pseudo) phenotypes for both traits.

Genome-wide association study

Genotype quality control was performed considering minor allele frequency (MAF) higher than 0.02 and call rate of 0.95 (SNP) and 0.90 (animal). Mitochondrial, X/Y and unmapped chromosomes were also excluded from analyses. After quality control, 463,539 SNPs remained for PP14 and 464,081 SNPs for SC. The genomic best linear unbiased (GBLUP) method was chosen for genomic prediction analysis, under a Bayesian approach, using BLUPF90 software (Mizstal *et al.,* 2002). A Markov chain with length 250,000 was created, considering a burn-in period of 50,000 and thinning intervals of 100 samples. Thus, inference was performed based in a posterior distribution containing 2,000 samples. The model considered to estimate SNP marker effects for both traits studied was the following: $y = 1\mu + Zg + e$, where y is the deregressed estimated breeding value for the trait, μ is the fixed effects vector (overall mean), g is the vector of random marker effects, e is the random residual effect and Z is the incidence matrix for random effect in g.

Rather than using effects of individual markers, the proportion of variance explained by non-overlapping 1Mb genomic windows comprising all the marker effects in that region were used for inference in the genome-wide association. The 10 windows explaining higher proportions of the total genetic variance were considered for the gene search analysis. Annotated genes in these regions were retrieved from Ensemble Genes 87 Database using Biomart software (Aken *et al.,* 2016) for both traits.

Results and Discussion

The ten windows responsible for the highest percentage of the genetic variance for scrotal circumference (SC) were located in chromosomes 1, 7, 10, 11, 14, 16 and 22, explaining a total of 6.26% of the total genetic variance (Figure 1, Table 1).

Table 1. Information on the top ten SNP windows responsible for the higher percentage of variance of the total genetic variance for SC trait in Nellore cattle.

Chr	i_pos (Mb)	f_pos (Mb)	n_SNPs	var (%)

16	64.4	65.4	389	124.886
1	63.0	64.0	270	0.961
14	53.4	54.4	264	0.789
7	110.4	111.4	345	0.554
11	29.4	30.4	347	0.535
22	27.4	28.4	343	0.462
10	9.7	10.7	398	0.444
14	79.6	80.6	374	0.444
1	99.8	100.8	307	0.422
1	142.4	143.4	404	0.403

Chr: chromosome; i_pos: window initial position; f_pos: window finishing position; n_SNPs: number of SNPs; var: percentage of the total genetic variation explained by the window;

Some QTLs related to SC were near or within the windows reported in the present study (*Table 1*). The window in chromosome 1 (63-64 Mbp) is placed in a QTL region described by McClure *et al*. (2010) in Angus cattle. The region found in BTA 11 (29-30 Mbp) also located inside a QTL region recently described by Irano *et al*. (2016) in Nellore cattle. The window reported in BTA 14 (53-54Mb) was placed 3.7Mb away from a previously described QTL (McClure *et al.,* 2010) for SC.

The highest proportion of the genetic variation was attributed to a window located in BTA 16 (64.4-65.4 Mbp), in which is located TEDDM1 gene, responsible for the epidemic transmembrane 1. Analyses performed by Yamazaki *et al*. (2006) revealed a significant increase in the mRNA expression of this gene in mouse at puberty. The SOCS5 gene found within the BTA 11 window (29.4-30.4-Mbp) is related to follicle activation and development (Keightley *et al.*, 2008). Other candidate genes were detected within the reported regions (UPK1B, MAN2A1, MSH2, EFNA5, MSH6, JMY, FER, CSMD3, PDZRN3, EPCAM, PAPD4, HOMER1) and had their functions associated to the following terms: celular adhesion, cellular multiplication, cellular signaling, neural function, cellular division, cellular differentiation and regulation of biological processes. Those may be affecting SC in males and could also be influencing reproductive performance in Nellore females. Thus, it gets evident that some genes associated to SC have a clear association to female reproduction aspects.

The top ten windows responsible for the highest percentage of the genetic variance for the probability of pregnancy at 14 months (PP14) were located in chromosomes 5, 6, 10, 12, 17, 20 and 23, explaining 4.38% from the total genetic variance of the trait (Figure1, Table 2).

FIGURE 1

Table 2. Information on the top ten SNP windows responsible for the higher percentage of variance of the total genetic variance for PP14 trait in Nellore cattle.

Chr	i_pos (Mb)	f_pos (Mb)	n_SNPs	var (%)
6	80.8	81.8	365	0.647

12	82.5	83.5	398	0.530
20	40.3	41.3	297	0.493
10	12.0	13.0	380	0.439
18	15.4	16.4	290	0.433
17	11.8	12.8	352	0.415
23	49.3	50.3	300	0.395
6	53.9	54.9	302	0.381
6	66.5	67.5	300	0.331
5	21.0	22.0	319	0.318

Chr: chromosome; i_pos: window initial position; f_pos: window finishing position; n_SNPs: number of SNP ; var: percentage of the total genetic variation explained by the window;

The region found in BTA 23 is located within a QTL related to age at puberty in Brahman and Tropical Composite cattle (Hawken *et al.,* 2012). The window placed in BTAs 18, 6 and 5 were relatively close to QTLs previously associated to age at puberty in Jersey and Limousin (Morris *et al.*, 2009) breeds, Brahman and Tropical Composite cattle (Hawken *et al.*, 2012), and also to heifer pregnancy in Nellore breed (Irano *et al.,* 2016), located 0.8, 3.9 and 3.9 Mbp away from the specified QTLs, respectively.

In BTA 12, the SLC10A2 was described in lactating cows for having a greater expression in granulosa cells of the dominant follicle (Sanchez *et al*., 2014). Also in the window reported located in BTA 20 (40.3-41.3Mbp), it was found NPR3, a receptor commonly found in cumulus cells (Cesaro *et al.,* 2015). Other candidate genes (Y-RNA, ECI2, LSM6, TARS, PCDH7, NETO2, SLC10A2, PHKB, LYRM4, ECI2, PP1R3G, GABRA4, GABRA1) had their biological functions associated to the following terms: cellular replication, metabolic processes, tissue development, neural system development and may also be influencing this trait.

There were no overlapping regions when comparing the top 10 windows responsible for the higher proportion of the genetic variance for both PP14 and SC. It gets clear that PP14 is controlled by a great number of loci throughout the bovine genome loci, and is probably affected by many indirect factors linked to puberty as, for example, factors associated to the corporal development and ability (of the female) to adapt to harsh environments. At the same time, SC seems to be more intimately and directly linked to the male reproduction process itself.

Conclusion

The genome-wide association study allowed the identification of major regions affecting both the probability of pregnancy at 14 months (PP14) and scrotal circumference (SC), utilized as puberty and fertility indicators in Nellore females and males, respectively. The absence of overlapping regions between the top 10 marker windows for both traits suggest that precocity in male and female is not strongly associated and, thus, should be independently considered as selection criteria.

List of References

Aken, B.L., S. Ayling, D. Barrell, L. Clarke, V. Curwen, S. Fairley, J. Fernandez-Banet, K. Billis, C. Garcia-Giron, T. Hourlier, K. L. Howe, A. K. Kahari, F. Kokocinski, F. J. Martin,

D. N. Murphy, R. Nag, M. Ruffier, M. Schuster, Y. A. Tang, J.-H. Vogel, S. White, A. Zadissa, P. Flicek, & S. M. J. Searle, 2016. The Ensembl Gene Annotation System. Database (Oxford). 2016:baw093. doi:10.1093/database/baw093.

CFM, 2017. Sumário de touros Nelore, Agropecuária CFM.

De Cesaro, M.P., M.P. Macedo, J.T. Santos, P.R. Rosa, C.A. Ludke, V.B. Rissi, B.G. Gasperin & P.B. Gonçalves, 2015. Natriuretic peptides stimulate oocyte meiotic resumption in bovine. Animal Reproduction Science 159:52–59.

Garrick, D., J. Tayor & R. Fernando, 2009. Deregressing estimated breeding values and weighting information for genomic regression analyses. Genetics Selection Evolution, 41:55.

Hawken, R.J., Y.D. Zhang, M.R.S. Fortes, E. Collis, W.C. Barris, N.J. Corbet, P.J. Williams, G. Fordyce, R.G. Holroyd, J.R.W. Walkley, W. Barendse, D.J. Johnston, K.C. Prayaga, B. Tier, A. Reverter & S.A. Lehnert, 2012. Genome-wide association studies of female reproduction in tropically adapted beef cattle. J. Anim. Sci. 90(5): 1398-410.

Irano, N., G.M. de Camargo, R.B. Costa, A.P. Terakado, A.F. Magalhães, R.M. Silva, M.M. Dias, A.B. Bignardi, F. Baldi, R. Carvalheiro, H.N. de Oliveira & L.G. de Albuquerque, 2016. Genome-Wide Association Study for Indicator Traits of Sexual Precocity in Nellore Cattle. PloS one 11(8): e0159502.

Keightley, R., E. McLaughlin, S.D. Roman, R.L. Robker & D.L. Russell, 2008. Suppressors of cytokine signalling (SOCS): roles in ovarian follicle activation. Reproduction, Fertility and Development 20, 68-68.

McClure, M.C., N.S. Morsci, R.D. Schnabel, J.W. Kim, P. Yao, M.M. Rolf, S.D. McKay, S.J. Gregg, R.H. Chapple, S.L. Northcutt & J.F. Taylor, 2010. A genome scan for quantitative trait loci influencing carcass, post-natal growth and reproductive traits in commercial Angus cattle. Anim Genet 41 (6): 597-607.

Misztal, I., S. Tsuruta, T. Strabel, B. Auvray, T. Druet & D. H. Lee. 2002. BLUPF90 and related programs (BGF90). Proc. 7th World Congr. Genet. Appl. Livest. Prod. 1–2.

Morris, C A; W.S. Pitchford, N.G. Cullen, A.K. Esmailizadeh, S.M. Hickey, D. Hyndman, K.G. Dodds, R.A. Afolayan & A.M. Crawford, A M; Quantitative trait loci for live animal and carcass composition traits in Jersey and Limousin back-cross cattle finished on pasture or feedlot. Animal genetics, 2009, 40(5): 648-54.

Sanchez, R., Y. Schuermann, L. Gagnon-Duval, H. Baldassarre, B.D. Murphy, N. Gevry, L.B. Agellon, V. Bordignon & R. Duggavathi, 2014. Differential abundance of IGF1, bile acids, and the genes involved in their signaling in the dominant follicle microenvironment of lactating cows and nulliparous heifers. Theriogenology 81:771–779.

Van Melis, M.H., J.P. Eler, G.J.M. Rosa, J.B.S. Ferraz, L.G.G. Figueiredo, E.C. Mattos & Oliveira H.N., 2010. Additive genetic relationships between scrotal circumference, heifer pregnancy, and stayability in Nellore cattle. J. Anim. Sci. 88:3809–3813.

Yamazaki, K., T. Adachi, K. Sato, Y. Yanagisawa, H. Fukata, N. Seki, C. Mori & M. Komiyama, 2006. Identification and characterization of novel and unknown mouse epididymis-specific genes by complementary DNA microarray technology. Biol Reprod 75: 462–468.

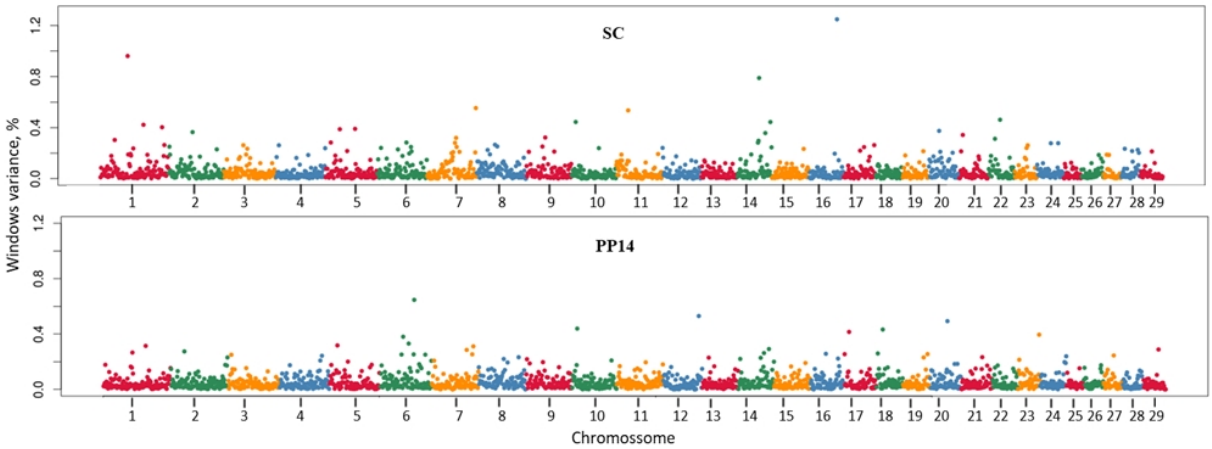

Figure 1. Manhattan plot showing the proportion of window variance (1Mb) accounted by genome locations for PP14 and SC in Nellore cattle.