Proceedings, 10th World Congress of Genetics Applied to Livestock Production

Genome-Wide Association Study on Long-Yearling Scrotal Circumference in Canchim Cattle

***M. E. Buzanskas*[1], D. A. Grossi[2], R. V. Ventura[2,3], T. C. S. Chud[1], I. Urbinati[1], S. L C. Meirelles[4], F. B. Mokry[5], F. S. Schenkel[2], L. C. A. Regitano[6], M. M. Alencar[6] and D. P. Munari[1]**

[1]Universidade Estadual Paulista (UNESP/FCAV), Jaboticabal, SP, Brazil, [2]Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada, [3]Beef Improvement Opportunities (BIO), Guelph, ON, Canada, [4]Federal University of Lavras (UFLA), Lavras, MG, Brazil, [5]Federal University of São Carlos (UFSCar), São Carlos, SP, Brazil, [6]Embrapa Southeast Livestock (CPPSE), São Carlos, SP, Brazil

ABSTRACT: Genome-wide association studies provide valuable information for understanding the genetic control of complex traits in livestock. The goal of this study was to investigate the association of the BovineHD BeadChip SNP genotypes with estimated breeding values for long-yearling scrotal circumference adjusted to 420 days (SC420) in Canchim beef cattle. A total 435 SNPs were significantly associated with SC420 (10% chromosome-wise FDR), of which 30 were located in genes on chromosomes 5, 13, and 14, including *HEY1*, *PLCG1*, *PAG1*, *ZFHX4*, *PEX2*, *FABP5*, *FABP12*, *MED30*, and *TRHR* genes. These genes play a role in biological processes related to reproduction, fat deposition, and hormonal systems development. Future studies targeting these regions and genes could provide better understanding of the genetic architecture of reproduction traits in Canchim cattle.

Keywords:
animal breeding
candidate genes
Canchim breed

Introduction

Reproduction traits are traditionally included in selection criteria of beef cattle breeding programs due to their great economic importance for breeders and beef producers. Scrotal circumference is indicative of reproductive potential in bulls, because testis size is associated with production and quality of sperm, as well as production of sex hormones (Trocóniz et al. (1991)). This trait shows moderate to high heritability in Canchim cattle (Gianlorenço et al. (2003); Borba et al. 2011)) and it is commonly measured at weaning, yearling, and post-yearling ages. The genetic correlation between scrotal circumference and reproductive traits of females, such as age at first calving (Silva et al. (2000)) and stayability (Buzanskas et al. (2010)), is moderate and favorable.

Many efforts have been made through the years aiming to identify quantitative trait loci (QTL) for production, reproduction, and health traits in many species. Nowadays, with the technological advances in genotyping platforms, the use of single nucleotide polymorphism (SNP) markers in livestock is a reality. One of many applications of the SNP chip technology is known as genome-wide association study (GWAS), which aims to identify QTL/genomic regions associated with quantitative traits. If candidate QTL/regions are identified, genetic selection could focus on these QTL/regions that play a role in biological functions and pathways associated with the traits of interest (Zhang et al. (2011)). Thus, the aim of this study was to identify genomic regions associated with long-yearling scrotal circumference in Canchim cattle.

Materials and Methods

Data. The Illumina Bovine HD BeadChip was used for genotyping 194 males and 205 females, of which 285 were from Canchim breed (62.5% Charolais and 37.5% Zebu) and 114 from ñMAò genetic group (65.6% Charolais and 34.4% Zebu). The genotyped individuals were born between 1999 and 2005, and originated from seven farms in the Brazilian states of São Paulo and Goiás. Estimated breeding values (EBVs) for long-yearling scrotal circumference adjusted to 420 days of age (SC420) were provided by the Embrapa-Geneplus Beef Cattle Breeding Program.

Genotype quality control. SNPs with genotype calling score lower than 15% were treated as missing genotypes. Genotype quality control was applied to exclude SNPs with significant ($P<10^{-5}$) Hardy-Weinberg Equilibrium deviation; excess heterozygosity of 15%; minor allele frequency lower than 5%; and call rate lower than 90%. Animals with call rate lower than 90% were also excluded and only autosomal SNPs with known genome position were used. SNP positions were defined according to the UMD_3.1 bovine assembly map.

Association analyses. Genome-wide association analyses were carried out using the Generalized Quasi-Likelihood Score method (GQLS), proposed by Feng et al. (2010). In this method, a logistic regression is used to associate the EBVs (treated as a covariate) with genotypes (treated as response variable), accounting for the population structure by means of the pedigree-based relationships among animals.

In order to account for multiple comparisons, a chromosome-wise false discovery rate (FDR) of 10% was applied. The significant SNPs were mapped to their corresponding genes using the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and the Ensembl Genome Browser (http://www.ensembl .org/biomart) genome databases. Functional analysis of the mapped genes was performed by means of the UniProt website (http://www.uniprot.org/), which is a comprehensive and freely available resource of functional information.

Results and Discussion

A total of 786,799 SNPs and 399 animals were originally existent in the database and, after quality control, 672,778 SNPs and 392 animals remained for GWAS. A total of 435 SNPs were significantly associated with SC420 at a 10% chromosome-wise FDR. However, we focused on significant SNPs located within genes. A total of 30 SNPs, located on chromosomes 5, 13, and 14, were mapped to 22 genes and these genes were then submitted for functional analysis. In Table 1 the 30 significant within gene SNPs are listed. Figure 1 depicts the corresponding Manhattan plots for chromosomes 5, 13, and 14.

Information on the genes *SNORA42*, *SLC35E3*, *SNORA38 LOC789021*, *LOC781434*, and *FAM82B* was not available in the consulted databases. The genes *RAP1B (RAP1B*, member of *RAS oncogene family)*, *NUP107 (nucleoporin 107kDa)*, *NOV (nephroblastoma overexpressed gene)*, and *ABRA (actin-binding Rho activating protein)* are part of cellular activities, such as transport, proliferation, growth, and transcription coactivator, respectively. The molecular functions of *MRPS28 (mitochondrial ribosomal protein S28)* and *TPD52 (tumor protein D52)* genes are RNA and calcium ion binding, respectively. As for the *TNFRSF11B (tumor necrosis factor receptor superfamily)* gene, it participates in bone mineral density. In humans, polymorphisms in this gene are associated with osteoporosis (Vidal et al. (2006))

Located on chromosome 13, the *PLCG1* gene (*phospholipase C, gamma 1*) participates in cellular response to epidermal growth factor stimulus, *in utero* embryonic development, positive regulation of epithelial cell migration, and in the phospholipid catabolic process (Ji et al. (1997)). On chromosome 14, SNPs were located in the *HEY1* gene (*hairy/enhancer-of-split related with YRPW motif 1*), which participates in the process of angiogenesis and artery and osteoblast development (Fischer et al. (2007)). According to Carletti and Christenson (2009), there is a considerable increase in *HEY1* expression (angiogenesis) during the follicle to *corpus luteum* transformation in mouse. The *PAG1 (pregnancy-associated glycoprotein 1)* gene participates in proteolysis and is detected in maternal serum soon after embryo implantation (Xie et al. (1995)).

The *ZFHX4* (*zinc finger homeobox 4*) gene was reported as responsible for the molecular regulation of puberty in beef cattle and it is already known as a candidate gene for this trait (Fortes et al. (2011)). The literature also reports that SNPs in the *ZFHX4* gene were associated with milk yield and fat percentage traits in Holstein breed cattle (Kolbehdari et al. (2009)). The genes *MED30* (*mediator complex subunit 30*) and *TRHR* (*thyrotropin-releasing hormone receiver*) trigger hormonal processes that are associated with growth. The *MED30* gene participates in androgen receptor signaling pathway, stem cell maintenance, and positive regulation of transcription (Wang et al. (2002)); and the *TRHR* gene has a molecular function in thyrotropin-releasing hormone receptor activity, i.e. stimulating the release of thyroid hormones (Takata et al. (1998)). Studies involving humans and animals indicate that the thyroid hormone plays an important role in the development and function of the cardiovascular, nervous, immune and reproductive systems (Krassas (2007)).

The *PEX2 (peroxisomal biogenesis factor)* gene participates in bile acid biosynthesis, cholesterol homeostasis, fatty acid beta-oxidation and the regulation of cholesterol biosynthesis (Van Veldhoven (2010)). The genes *FABP5 (fatty acid binding protein 5)* and *FABP12 (fatty acid binding protein 12)* have molecular functions of transporter activity, lipid binding, and homeostasis of adipocytes (Michal et al. (2006); Liu et al. (2008)). Considering that cholesterol acts as a precursor of steroid hormones, such as testosterone, and that fat is an important feature for achieving sexual maturity; *PEX2*, *FABP5*, and *FABP12* are suggested as potential candidate genes for precocity in cattle.

Conclusion

We observed that the *ZFHX4* gene indeed plays a role on reproductive traits in beef cattle. New candidate genes (*HEY1*, *PLCG1*, *PAG1*, *PEX2*, *FABP5*, *FABP12*, *MED30*, and *TRHR*) for scrotal circumference were detected on chromosomes 5, 13, and 14. These genes have biological functions related to bovine growth and reproductive performance. Future studies targeting these genes/regions could provide better understanding of the genetic architecture of reproductive traits in Canchim cattle.

Acknowledgements

M. E. Buzanskas, I. Urbinati, and T. C. S. Chud would like to thank the São Paulo Research Foundation (FAPESP) (grantee n. 13/19335-2, 13/09050-0, and 12/21891-8). F. B. Mokry was supported by a fellowship from the Coordination for Enhancement of Higher Education Personnel (CAPES/PNPD) (grantee n. 02663/09-0). D. P. Munari, L. C. A. Regitano, and M. M. Alencar were supported by a fellowship from the National Council of Technological and Scientific Development (CNPq).

Literature Cited

Borba L. H. F., Rey F. S. B., Silva L. O. C., et al. (2011). Pesq. Agropec. Bras., 46:1570ï 1578.

Buzanskas M. E., Grossi D. A., Baldi F., et al. (2010). Livest. Sci., 132: 107ï 112.

Carletti M. Z., Christenson L. K. (2009). Reproduction 137:843ï 855.

Feng Z., Wong W. W. L., Gao X. et al. (2011). Ann. Appl. Stat. 5: 2109ï 2130.

Fischer A., Steidl C., Wagner T. U. et al. (2007). Circ. Res. 100:856ï 863.

Fortes M. R. S., Reverter A., Nagaraj S. H. et al. (2011). J. Anim. Sci., 89:1669ï 1683.

Gianlorenço V. K., Alencar, M. M., Toral, F. L. B. et al. (2003). R. Bras. Zootec. 32:1587-1593.

Ji Q. S., Winnier G. E., Niswender K. D. et al. (1997). Proc. Natl. Acad. Sci. U. S. A. 94:2999ï 3003.

Kolbehdari D., Wang Z., Grant J. R. et al. (2009). J. Anim. Breed. Genet. 126:216ï 227.

Krassas G. E. (2007). Clin. Endocrinol. 66:309ï 321.
Liu R. Z., Li X., and Godbout R. (2008). Genomics, 92:436ï 45.
Michal J. J., Zhang Z. W., Gaskins C. T. et al. (2006). Anim. Genet. 37:400ï 402.
Silva, A. M., Alencar, M. M., Freitas, A. R., et al. (2000). Rev. Bras. Zootec. 29:2223-2230.
Takata M., Shimada Y., Ikeda A. et al. (1998). J. Vet. Med. Sci. 60:123ï 127.
Trocóniz J. F., Beltrán J., Bastidas H. et al. (1991). Theriogenology, 35:815ï 826.
Van Veldhoven P. P. (2010). J. Lipid. Res. 51:2863ï 2895.
Vidal C., Brincat M., Xuereb Anastasi A. (2006). Maturitas 53:386ï 395.
Wang Q., Sharma D., Ren Y. et al. (2002). J. Biol. Chem. 277:42852ï 42858.
Xie S., Green J., Beckers J. F. et al. (1995). Gene 159:193ï 197.
Zhang Z., Ding X., Liu J., et al. (2011). J. Dairy Sci. 94: 3642ï 365

Table 1. Single nucleotide polymorphisms (SNPs) within genes significantly (10% chromosome-wise FDR[1]) associate with long-yearling scrotal circumference.

SNPs	Gene Symbol	Chr: Pos	p-value
rs136466664 *	*SLC35E3*	5: 45.24	2.91E-4
rs110291739 À		5: 45.26	6.37E-5
rs110572792 ÿ	*NUP107*	5: 45.27	6.37E-5
rs134370690 §,ÿ	*NUP107, SNORA38*	5: 45.28	9.24E-5
rs109284796 §,À		5: 45.28	5.66E-6
rs110852214 §,À		5: 45.29	3.89E-4
rs133990240 ÿ	*RAP1B*	5: 45.35	1.42E-6
rs110261691 ÿ		5: 45.35	1.42E-6
rs133273718 Á	*PLCG1*	13: 70.48	2.11E-5
rs42142739 Á	*ZFHX4*	14: 41.98	1.19E-3
rs137442228 #	*PEX2*	14: 42.33	8.39E-4
rs132803686 ÿ	*HEY1*	14: 45.12	2.95E-4
rs136891270 ÿ		14: 45.12	2.83E-4
rs134553723 ÿ,Á	*MRPS28, TPD52*	14: 45.49	1.21E-4
rs136546448 ÿ	*TPD52*	14: 45.49	1.21E-4
rs110246732 À		14: 45.53	1.82E-5
rs137291182 ÿ	*PAG1*	14: 46.33	1.64E-6
rs133930486 Á	*FABP5*	14: 46.64	3.72E-4
rs137684819 ÿ		14: 46.65	1.33E-4
rs43765465 Á	*FABP12*	14: 46.89	7.68E-5
rs135988903 ÿ	*NOV*	14: 47.00	1.60E-4
rrs133657412 §,Á	*TNFRSF11B,SNORA42*	14: 47.43	2.27E-4
rs137422799 §,ÿ		14: 47.44	2.27E-4
rs136481210 §,ÿ		14: 47.44	2.27E-4
rs135065691 ÿ	*MED30*	14: 48.93	8.27E-4
rrs134994711 ÿ	*LOC789021*	14: 50.16	4.85E-4
rs137450084 ÿ	*LOC781434*	14: 50.60	1.73E-4
rs133457508 Á	*TRHR*	14: 57.52	2.27E-4
rs136790655 Á	*ABRA*	14: 59.96	2.52E-4
rs134287222 ÿ	*FAM82B*	14: 78.59	8.74E-4

[1]FDR= False discovery rate, Chr= chromosome, Pos = position in Mb, *3ô UTR variant, ÀUpstream variant, ÿDownstream variant, §Intron variant, #5ô UTR variant.

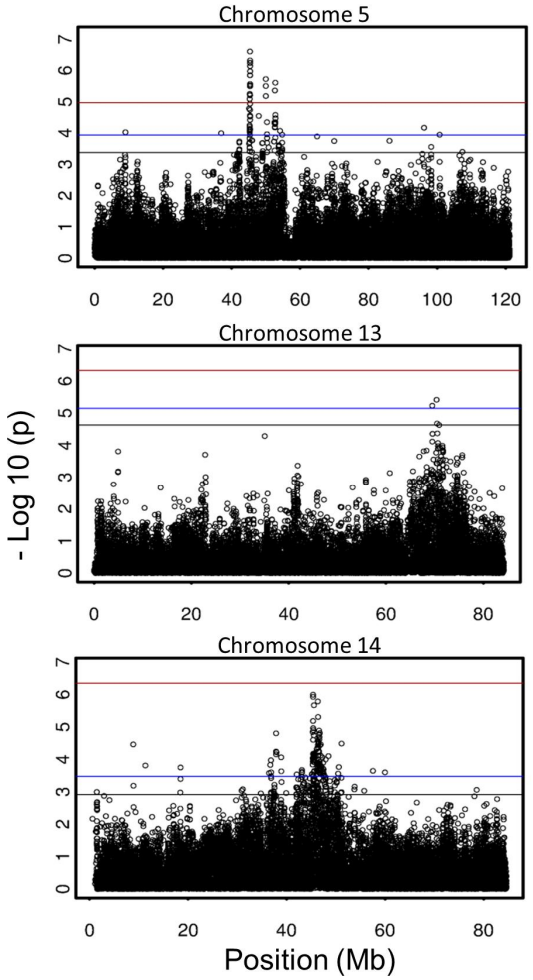

Figure 1. Manhattan plot of p-values for long-yearling scrotal circumference. Significance levels were determined by false discovery rate (FDR) at 1% (red line), 5% (blue line), and 10% (black line).