GENOME-WIDE MAPPING OF LOCI AFFECTING CARCASS PREDICTIVE TRAITS IN BRAZILIAN NELLORE CATTLE

Anirene G. T. Pereira[1*], Yuri T. Utsunomiya[2], Márcio da S. Costa[3], José L.R. Sarmento[3], Curtis P. Van Tassell[4], Tad S. Sonstegard[4], José Fernando Garcia[5], Carmen J. Contreras-Castillo[1]

[1] Escola Superior de Agricultura "Luiz de Queiroz", USP, Piracicaba, Brazil
[2] Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, Brazil
[3] Universidade Federal do Piauí, UFPI, Teresina, Brazil
[4] Bovine Functional Genomics Laboratory, ARS-USDA, Beltsville, USA
[5] Faculdade de Medicina Veterinária de Araçatuba, UNESP, Araçatuba, Brazil
*anirene_eng.alimentos@hotmail.com

Abstract – Weight measurements and visual evaluations of conformation, precocity and muscling (CPM) are routinely employed in beef cattle selection for carcass yield in Brazil. These traits are inexpensive and effortless to measure, and present moderate heritability. The present study aimed at mapping loci associated with weight and CPM traits in Brazilian Nellore cattle. Seven hundred eighty eight animals had estimated breeding values available for the following traits: birth weight (BW), pre-weaning gain (WG), post-weaning gain (PWG), conformation at weaning (CW), conformation at yearling (CY), precocity at weaning (PW), precocity at yearling (PY), muscling at weaning (MW), and muscling at yearling (MY). The same animals were genotyped with the Illumina® BovineHD BeadChip assay. Both single and multi-trait association analyses highlighted the *PLAG1* chromosome region as a key regulator of traits that are predictive of carcass weight.

I. INTRODUCTION

Carcass traits are of paramount economic importance in beef cattle. In Brazil, the carcass retail price is essentially determined by its weight. Variation in growth rate and weight gain is partially explained by genetic differences among animals, making selection and breeding important factors in the competitiveness of beef cattle production in the country (1).

Carcasses with higher dressing percentage can improve producers' revenues as their remuneration is based directly on carcass weight. Moreover, carcasses with higher yield of commercial cuts decrease fabrication costs per unit of product in packing plants. Weight measurements, as well as visual scores of conformation, precocity and muscling (CPM) are routinely employed in beef cattle selection for carcass yield in Brazil. These traits are inexpensive and effortless to measure, and present moderate heritability (2). The inclusion of visual evaluation by scores or grades in breeding programs is presented as an alternative for improving meat yield and associated carcass traits, as well as precocity of carcass finishing (3).

As per the heritability estimates, Boligon *et al.* (4) found: 0.16 ± 0.02 (conformation at weaning - CW); 0.20 ± 0.02 (precocity at weaning - PW); 0.19 ± 0.02 (muscling at weaning - MW); 0.24 ± 0.02 (conformation at yearling - CY); 0.31 ± 0.02 (precocity at yearling - PY); 0.32 ± 0.02 (muscling at yearling - MY) and 0.46 ± 0.04 (mature weight - MW).

Therefore, this study aimed at mapping chromosome segments affecting weight and CPM traits in Brazilian Nellore cattle.

II. MATERIALS AND METHODS

Genotypes and phenotypes

A previously reported dataset of 788 influential Nellore sires genotyped with the Illumina® BovineHD BeadChip assay (5, 6, 7) was used in the present study. These animals had phenotypic data available for the following traits: birth weight (BW), pre-weaning gain (WG), post-weaning gain (PWG), conformation at weaning (CW), conformation at yearling (CY), precocity at weaning (PW), precocity at yearling (PY), muscling at weaning (MW), and muscling at yearling (MY). Estimated breeding values for these traits were obtained from routine genetic

evaluations using performance and pedigree data from the *Aliança Nelore* database (8).

Genotyping and data filtering

Only autosomal markers satisfying the following inclusion criteria were used in the downstream analyses: 1) minor allele frequency (MAF) greater than 0.02, 2) Fisher's exact test *p*-value for Hardy-Weinberg Equilibrium greater than 1 x 10^{-5} and 3) call rate of at least 0.98. All genotyped samples presented call rate greater than 0.9, thus no threshold was applied to filter individuals by this criterion. These procedures, as well as others described later, were performed using customized functions and the *base* and the *GenABEL v1.8* (9) packages in *R v3.0.1* (10).

Single-trait association analysis

We adapted a mixed model approach, namely *Fast Association Score Test-based Analysis* (FASTA) (11), to test each SNP for associations. The same single-trait model was applied across traits: y ~ mean + SNP + animal + error. The animal and error terms were modeled as random effects, whereas markers were fitted as fixed effects. Variance components were estimated by restricted maximum likelihood, and marker effects and test statistics obtained via generalized least squares. The heterogeneity of variance in dEBVs was controlled by using linear transformations of the design matrices rotated on a weight matrix.

Multi-trait association analysis

Three different approaches were applied in order to identify putative markers affecting multiple CPM and weight traits. The first method was originally described by Bolormaa *et al.* (12), and consists in the computation, for each SNP i, of the test statistic $t_i' C^{-1} t_i$, where t_i is a j x 1 vector of signed t-values across k traits, and C is the j x j correlation matrix of t-values across all m SNPs. Under the null hypothesis, this test statistic is distributed as χ^2 with k degrees of freedom.

The second approach consisted in the meta-analysis of *P*-values under the framework of the widely known Stouffer method (13). The rationale of this analysis is: for any given trait, SNP *p*-values are uniformly distributed in the interval between 0 and 1 under the hypothesis of no association. This distributional property allows for the use of the inverse Gaussian density to produce Z-scores for each combination of SNP i and trait j as $Z_{ij} = \phi^{-1}(1 - p_{ij})$, where p_{ij} is the *p*-value. These Z-transformed *p*-values can then be averaged and standardized to produce a multi-trait test statistic:

$$\frac{\sum_{j=1}^{k} Z_{ij}}{\sqrt{k + 2C}}$$

where C is the sum of all pairwise Pearson's product-moment correlations between traits computed from the vectors of Z-scores. Under the hypothesis of no association, the combined scores are distributed as $N(0,1)$, and joint *p*-values can be computed from the normal cumulative density function.

The last approach was based on an eigendecomposition of the phenotypic covariance matrix. Let Y be the n x k matrix containing n observations for k traits. Prior to any analysis, each column of Y is rotated on its respective weight matrix in order to account for the heterogeneity of variance among observations. Then, the columns of Y are centered and scaled by their respective mean and standard deviation. The covariance matrix is then computed as $\sum = YY'$, and a singular value decomposition is applied as $\sum = UDU'$. As the first column of the matrix of eigenvectors U contains the orthogonal variable that explains the largest proportion of the phenotypic variance across all traits, this synthetic variable was used as a pseudo-phenotype in a single-trait Genome-Wide Association (GWA) analysis, as described before.

False discovery rate

The expected number of false discoveries among the SNPs declared significant was calculated as: $FDR = \alpha m / s$, where m is the number of markers being tested, α is the

significance level, and s is the number of markers with $p < \alpha$. We adopted a significance threshold of $\alpha = 1 \times 10^{-4}$.

III. RESULTS AND DISCUSSION

Association analysis

After data filtering, a total of 500,263 SNPs were included in the association analyses. Inflation was close to 1 for all single-trait analyses, indicating that no important confounding effects were neglected. The Stouffer method presented the lowest FDR across all multi-trait approaches (6.4%).

Figure 1 shows the Manhattan plot portraying genome-wide -$\log_{10}$(p-values) for the meta-analysis of estimated breeding values for all nine traits analyzed.

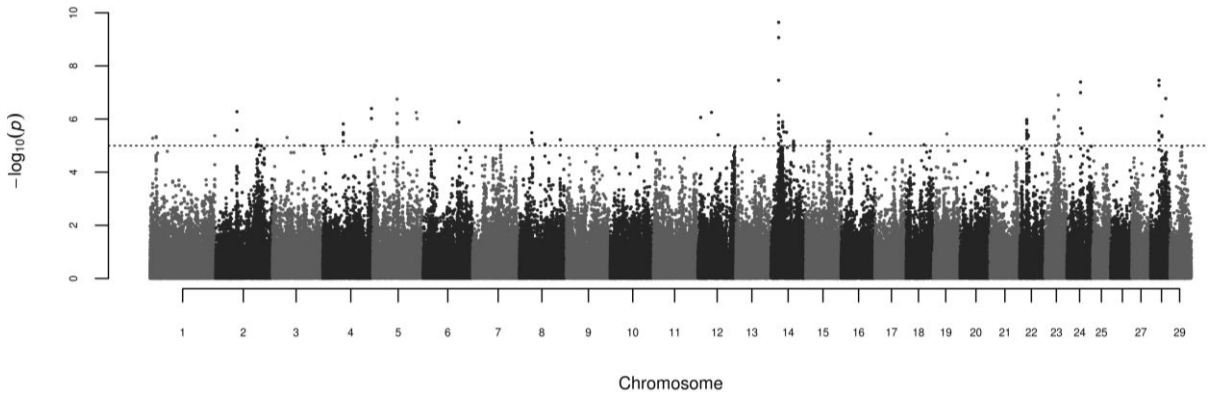

Fig 1. Manhattan plot of genome-wide -log10(***p*-**values) for the meta-analysis of estimated breeding values for birth weight, weaning gain, post-weaning gain, conformation at weaning, conformation at yearling, precocity at weaning, precocity at yearling, muscling at weaning and muscling at yearling. The horizontal solid line represents the significance level adopted ($\alpha = 1 \times 10^{-4}$, FDR = 6.4%)

The strongest association signal detected was found on chromosome 14, mapping to the chromosome domain encompassing *PLAG1*. This genomic region has been previously described to be associated with stature, weight gain and insulin-like growth factor I (Igf1) levels in cattle (14).

This quantitative trait locus (QTL) surrounding the transcription factor *PLAG1* was first associated with height and weight in Holstein-Jersey crosses (14), being also reported in Japanese black (15). As described by Fortes et al. (16), the work carried out with Tropical Composite and Brahman cattle was the first to establish the association between this QTL and Igf1 levels. Subsequently, the *PLAG1* region was demonstrated to be associated with birth weight and scrotal circumference in Nellore cattle (5,6).

Our findings are supported by the reports of Bollormaa *et al.* (12) and Fortes *et al.* (17), who presented evidence that the chromosome 14 QTL is pleiotropic, i.e., affects several growth and reproductive traits. Here, we present evidence that the *PLAG1* domain also affects multiple traits that correlate with carcass yield in Nellore cattle.

IV. CONCLUSION

The results presented here support the genomic region harboring *PLAG1* as a key regulator of traits that are predictive of carcass weight. The investigation of the interplay between *PLAG1* and Igf1, as well as associations between this QTL and direct carcass measurements, is needed in order to dissect the genetic component explaining differences in meat yield in Nellore cattle.

ACKNOWLEDGEMENTS

DeltaGen for animal samples and phenotypic data. Sao Paulo Research Foundation (FAPESP) – process 2013/02552-0, 2011/16643-2 and 2010/52030-2; and Brazilian Council for Research (CNPq) - process 560922/2010-8 and 483590/2010-0, for the financial support of this work.

REFERENCES

1. Kim, B.S.; Kim, S.C.; Park, C.M.; Lee, S.H.; Cho, S.H.; Kim, N.K.; Jang, G.W.; Yoon, W.H.; Yang, B.S.; Hong, S.H.; Seong, H.H.; Choi, B.H. (2013). Genetic polymorphism of the bovine NOV gene are significantly associated with carcass traits in Korean cattle. Asian Australasian Journal of Animal Science 26: 780-787.
2. Cancian, P.H.; Gomes, R.C.; Manicardi, F.R.; Ianni, A.C.; Bonin, M.N.; Leme, P.R.; Leme, P.R.; Silva, S.L. (2014). Correlations of visual score, carcass traits, feed efficiency and retail product yield in Nellore cattle. Scientia Agricola 71:17-22.
3. Araujo, R.O.; Rorato, P.R.N., Weber, T.; Everling, D.M.; Lopes, J.S.; Dornelles, M.A. (2010). Genetic parameters and phenotypic and genetics trends for weight at weaning and visual scores during this phase estimated for Angus-Nellore crossbred young bulls. Revista Brasileira de Zootecnia 39:2398-2408.
4. Boligon, A.A.; Mercadante, M.E.Z.; Albuquerque, L.G. (2011). Genetics association of conformation, finishing precocity and muscling visual score with mature weight in Nelore cattle. Livestock Science 135:1-6.
5. Utsunomiya, Y.T.; Do Carmo, A.S.; Carvalheiro, R.; Neves, H.H.R.; Matos, M.C.; Zavarez, L.B.; O'Brien, A.M.P.; Solkner, J.; McEwan, J.C.; Cole, J.B.; Van Tassel, C.P.; Shenkel, F.S.; Da Silva, M.V.G.B.; Neto, L.R.P.; Sonstegard, T.S.; Garcia, J.F. (2013). Genome-wide association study for birth weight in Nellore cattle points to previously describe orthologous genes affecting human and bovine weight. BMC Genetics
6. Utsunomiya, Y.T.; Carmo, A.S.; Neves, H.H.R.; Carvalheiro, R.; Matos, M.C.; Zavarez, L.B.; Ito, P.K.R.K.; O'Brien, A.M.P.; Solkner, J.; Poto-Neto, L.R.; Schenkel, F.S.; McEwan, J.; Cole, J.B.; da Silva, M.V.G.B.; Van Tassel, C.P.; Sonstegard, T.S.; Garcia, J.F. (2014). Genome-wide mapping of loci explaining variance in scrotal circumference in Nellore cattle. Plos One 9:1-9.
7. Neves, H.H.R.; Carvalheiro, R.; O'Brien A.M.P; Utsunomiya, Y.T.; do Carmo, A.S.; Scjenkel, F.S.; Solkner, J.; McEwan, J.; Van Tassel, C.P.; Cole, J.B.; da Silva, M.V.G.B.; Queiroz, S.A.; Sonstegard, T.S.; Garcia, J.F. (2014). Accuracy of genomic predictions in *Bos indicus* (Nellore) cattle. Genetics Selection Evolution 46: 1-13.
8. Aliança (2012) Sumário de touros 2012 Aliança Nelore. GenSys Consultores Associados.
9. Aulchenko, Y.S.; Ripke, S.; Isaacs, A.; Van Duijin, C.M. (2007). GenABEL: an R library for genome-wide association analysis. Bioinformatics 23:1294-1296.
10. R Development Core Team. 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org).
11. Chen, W.M.; Abecasis, R.G. (2007). Family-Based Association Tests for Genomewide Associaation Scans. The American Jorunal of Human Genetics 81:913-926.
12. Bolormaa, S.; Pryce, J.E.; Reverter, A.; Zhang, Y.; Barendse, W.; Kemper, K.; Tier, B.; Savin, K.; Hayes, B.J.; Goddard, M.E. (2014). A multi-trait, meta-analysis for detecting pleiotropic polymorphisms for stature, fatness and reproduction in beef cattle. Plos Genetics 10:1-13.
13. Whitlock, M.C. (2005). Combining probability form independent tests: the weighted Z-method is superior to Fisher approach. Jornal of Evolutionary Biology 18:1368-1373.
14. Karim, L.; Takeda, H.; Lin, L.; Druet, T.; Arias, J.A.C.; Cambisano, N.; Davis, S.R.; Farnir, F.; Grisart, B.; Harris, B.R.; Keehan, M.D.; Littlejohn, M.D.; Spelman, R.J.; Goerges, M.; Coppieters, W. (2011). Variants modulating the expression of a chromosome domain encompassing PLAG1 influence bovine stature. Nature Genetics 43:405-415.
15. Nishimura, S.; Watanabe. T.; Mizoshita, K.; Tatsuda, K.; Fujita, T.; Watanabe, N.; Sugimoto, Y.; Takasuga, A. (2012). BMC Genetics 13:1-12.
16. Fortes, M.R.S.; Reverter, A.; Kelly, M.; McCulloch, R.; Lehnert, S.A. (2013). Genome-wide association study for inhibin, luteinizing hormone, insulim-like growth factor 1, testicular size and semen traits in bovine species. Andrology 1:644-650.
17. Fortes, M.R.S.; Kemper, K. Sasazaki, S.; Reverter, A.; Pryce, J.E., et al. (2013) Evidence for pleiotropism and recent selection in the PLAG1 region in Australian Beef cattle. Animal Genetics 44:636-647.