Genome wide association study between copy number variation regions with marbling score in Nelore cattle

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ABSTRACT: The present study aimed to identify the association between copy number variation regions (CNVRs) with marbling score in Nelore cattle. Data from 737 Nelore bulls finished in feedlot were used. The animals were genotyped using the BovineHD BeadChip. A wide association analysis using adjusted phenotypic values were performed applying a general linear model. There were 10 CNVRs with a MAF threshold above 0.05. There was a CNVR located on chromosome 12 and detected in 227 animals, significantly associated (p<0.05) with marbling score.

Keywords: CNVR; Meat quality; Zebu cattle; GWAS

Introduction

In beef cattle, the carcass and meat traits have a great importance in determining the price and access to new markets. The meat quality is associated to its organoleptic traits, such as color, flavor, juiciness and tenderness. These organoleptic traits are influenced by the subcutaneous fat coverage and intramuscular fat content. The intramuscular fat content (IMF) is responsible for meat marbling, which is one of the determining factors of meat texture, favoring chewing and flavor. The intramuscular fat deposition depends of the breed, age of the animal, feeding and management. In general, taurine breeds have higher levels of intramuscular fat content than zebu breeds (Burrow et al., 2001).

The copy number variation (CNV) is characterized as a segment of DNA that has 1 kb or more in length and is present in a variable number of copies compared to the reference genome (Feuk et al., 2006). It can also be described by the breakpoint loci (points of beginning and ending), simple length copies and number of copies. The regions of copy number variations (CNVRs) are obtained by the concatenation of CNVs in multiple samples. Freeman et al. (2006) showed that the CNVs are important to explain the phenotypic variability of the productive traits and susceptibility to disease. In these sense, CNV regions associated with feed efficiency in dairy cattle have been described by Hou et al. (2012). Therefore, we aimed to identify the association between CNVRs with marbling score in Nelore cattle.

Materials and Methods

A total of 737 Nelore bulls were finished in feedlot conditions (minimum period of 90 days) and slaughtered at approximately two years of age. The carcasses were chilled for a period of 48 hours. During boning, a sample of one inch (2.54 cm) of Longissimus dorsi muscle was collected between the 12 and 13th ribs from the left carcass side. A visual grading scale (USDA - Quality and Yield Grade, 1997), varying from 0 to 9, was used to determine the marbling score. A panel with over 777,000 SNPs in the BovineHD BeadChip (High-Density Bovine BeadChip) was used to genotype the animals. Samples with a call rate below 90% were excluded from the analyses.

The PennCNV algorithm (Wang et al., 2007), which incorporates multiple information sources from genotyping data, was used for CNV detection. The parameters used for estimating the CNVs were: Log R Ratio (LRR) and B Allele Frequency (BAF). For quality control, samples with standard deviation values for LRR <0.30, BAF derived as <0.05 and waving factor<0.01, were eliminated. The individual calls originated from the PennCNV software were grouped into CNVRs by the CNVRuler software (Kim et al., 2012). The recurrence parameter equal to 0.1 (areas with low density are excluded, < 10% of CNVs, to compose an estimated end region) was used, which leaves a more robust delimitation of the beginning and end of regions. The “Gain/Loss separated regions” option was additionally used, which compiles the region based on the genotype (gain or loss of copy number) instead of composing regions ignoring the event type.

A wide association analysis using adjusted phenotypic values were performed applying a general linear model. The marbling score records were adjusted for the fixed effects of farm and year of birth, and management groups at birth, weaning and yearling. The model included the state of CNVR (loss; gain or mixed) as a fixed effect and the animal age at slaughter as a covariate (linear and quadratic effects). The CNVRs with minor allele frequency (MAF) less than 0.05 were excluded.
Results and Discussion

The estimated mean for marbling score was 3.0, varying from 1.5 to 4.0. After applied the CNVRuler software, a total of 4,504 CNV regions were identified in the autosomal chromosomes. These 4,504 CNV regions include 1216 loss, 2464 gain and 824 both (loss and gain within the same region) events. The CNVR mean length size was 36.6 kb, varying from 0.65 to 1,487 kb.

There were 10 CNVRs with a MAF threshold above 0.05, thus these CNV regions were utilized for the wide association analyses with marbling score. There was a CNVR located on chromosome 12, beginning and ending at position 73,657,824 bp and 73,666,963 bp, respectively, and detected in 227 animals, significantly associated (p<0.05) with marbling score. This CNVR is within an intron 22 of the ATP-binding cassette gene, sub-family C (CFTR/ MRP), member 4-like (ABCC4). This gene, among other functions, plays an important role in the transport of bile from the liver to the blood in mice (Liaset et al., 2009). The bile has the function of separate the lipids into smaller structures in the digestive tract to facilitate absorption by the small intestine. Defects in this gene can lead to defects in the protein and its biological function, decreasing the absorption of fat and, probably, the deposition of intramuscular fat.

Moreover, Lee et al (2013) analyzing this gene in Hanwoo cattle, found a very large number of NS/SS/I polymorphisms (Non synonymous SNPs, splice-site variants, and coding indels) reaching a hypothesis that this gene evolved into a gene with multiple copies for environment adaptation. In order to validate if the copy of intron 22 or any other gene polymorphism (signalized by the CNV in intron 22) are the responsible for the genetic/phenotypic influence, this region should be studied using molecular genetics tools.

Recently, Nalaila et al (2012) found a SNP on chromosome 12 (38,324,078 bp) that is associated with fat carcass grade in Angus x Charolais steers. Mizoguchi et al (2010), in a gene expression trial, found a gene (HMGB1) (chr12:30,535,052-30,538,977 bp) differently expressed in adipocytes of Japanese Black cattle. McClure et al. (2010) working with two commercial Angus populations in order to identify QTLs (Quantitative Trait Loci) for 14 productive and meat traits, reported a QTL on chromosome 12 (20,840,000 bp) for marbling. However, the location of these markers is far from the CNV region found in this study, suggesting that is a new QTL for marbling in Nelore cattle.

Conclusion

There is a region at the chromosome 12 with variations in the number of copies associated with marbling score in Nellore cattle. The marbling is a trait to be improved in zebu cattle, since this trait has impact on meat quality and consumer preferences, thus the CNVRs can be used as tools to genetically improve this trait.

Literature Cited

Liaset et al. (2009). Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids.