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Genome Wide CNVs analysis to identify variants associated with coat color in Gyr breed[1]

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Resumo: Diversos estudos reportaram CNVs envolvidos com a cor da pelagem em bovinos taurinos, mas um número muito pequeno deles explora o papel dessas variantes estruturais desse fenótipo em animais zebuínos. Sendo assim, o presente estudo objetivou identificar os CNVs associados aos padrões de cor da pelagem mais comuns em animais da raça Gir. Um estudo de associação ampla do genoma do tipo caso/contole foi realizado utilizando CNVs identificados em 445 touros, com o intuito de verificar aqueles associados a quatro contrastes entre diferentes pelagens: Chita x Chitado, Chita x Monocromo, Gargantilha x Monocromo e Mouro x Monocromo. Foram identificados 60 CNVs significativamente associados e dois importantes genes candidatos (KIT e TYRO3), além de um QTL para a cor de pelagem, previamente reportados em animais taurinos. Os resultados do estudo sugerem que as mesmas regiões genômicas podem ser responsáveis por controlar a cor da pelagem em bovinos taurinos e zebuinos.

Palavras–chave: *bos taurus indicus, gwas,* KIT, TYRO3, variantes estruturais, tolerância ao calor

Abstract: There are several studies reporting CNVs involved with cattle coat color, but a very few number of them explore structural variants role in indicine cattle coat color pattern. Therefore, the present study aim to identify CNVs associated with the most commons patterns of coat color in Gyr animals. A case/control genome wide CNV analysis were performed on 445 Gyr bulls to identify CNVs associated with four different Gyr coat color contrasts: Chita x Chitado, Chita x Monochrome, Gargantilha x Monochromes and Mouro x Monochromes. Sixty CNV were significant associated among all contrasts. Two coat color important candidate genes (KIT and TYRO3) and two QTLs previously reported in taurine animals, overlapped with these CNVs. The study results suggests that the same genomic regions can be responsible to control coat color phenotype in taurine and indicine cattle.

Key word: *bos taurus indicus***,** gwas, KIT, TYRO3, structural variants, heat tolerance

Introduction

In early domesticated stocks, coat color was a variable trait. As one of the most important features related to animals visual identity, coat color became a trait under intense selection, which resulted in color patterns within breeds. Furthermore, coat color shows economic significance due to its potentially deleterious pleiotropic effects, and popularity differences, since some colors are more popular in certain parts of the world, attaining higher commercial prices .

According to Smith (1996), pigmentation in cattle is determined by the presence of functional melanocytes. Abnormal migration may be one of the reasons for piebaldism phenotype, which is the mixture of pigmented and unpigmented coat areas. Among taurine breeds, this phenotype is particularly common in Holstein and Simmental and has attracted the attention of scientific community because it is expected that animals with higher proportion of white coat absorb less solar radiation and are therefore better buffered against heat stress in tropical and subtropical environments. Several studies already have identified copy number variants involved with coat color in livestock. Copy number variants found on AGOUTI gene that causes white coat color in sheep (Bickhart and Liu, 2014), and KIT

gene, which is involved with coat spotting in several cattle breeds, have been considered as candidates to understand coat color expression (Reinsch et al., 1999).

Nowadays, few studies have been conducted in order to explore the structural variants responsible for this phenotype in indicine cattle, thus the present study aim to identify CNVs associated with the most common patterns of coat color in Gyr animals.

Material and Methods

A total of 445 Gyr bulls were genotyped using the Illumina BovineHD Genotyping BeadChip (Illumina Inc., San Diego, CA). Animals were classified according to their coat color patterns into five groups: Chitado, Chita, Gargantilha, Monochrome and Mouro. The Gyr coat color were used as qualitative phenotype for genome-wide study. The Log R Ratios (LRR) from 777,961 SNP probes generated by BovineHD Genotyping BeadChip were imported from the GenomeStudio software into Golden Helix SNP & Variation Suite (SVS) 8.3.0 (Golden Helix Inc., Bozeman, MT, USA) and a total of 695,818 SNPs were mapped onto the *Bos taurus* genome assembly UMD 3.1 within 29 autosomes chromosomes.

Previously to CNVs identification, a waviness effect correction were applied to LRR based on genome GC content. The copy number analysis module (CNAM) under the multivariate option was used to segment chromosomes with a maximum of 100 segments per window (10,000 markers), a minimum of 5 markers per segment, and a significance level of p = 0.01 for pairwise permutations (n = 1,000) as described by Xu et al., (2014).

A case/control study was performed to test the following contrasts between coat color patterns: A - Chita x Chitado (n = 75 x 249), B - Chita x Monochrome (n = 75 x 47), C - Gargantilha x Monochromes (n = 51 x 47) and D - Mouro x Monochromes (n = 22 x 47). A linear regression under the additive genetic model was employed to identify CNVs associated with each contrast. Significant CNVs were counted at FDR significance level of (p-value) < 0.05.

The database Variation gene 79 from Biomart (Ensembl) and cattle QTLdb were used to map significant CNVs overlapping genes and previously reported QTLs to functionally explore candidates genomic regions.

Results and Discussion

From 1461 unique CNVs detected, a total of 60 CNVs were significant considering all contrasts (Table1). The contrast D presented the higher number of significant CNVs (n = 51), followed by contrasts A (n = 7) and B (n = 2). The contrast C did not show any significant CNV. Gene content analysis demonstrated that CNVs overlap 734 genes, highlighting stem cell growth factor receptor c-Kit (KIT) and tyrosine-protein kinase receptor (TYRO3). These genes have already been reported as important candidates involved with coat color in taurine breeds.

The KIT gene is included in a region denominated *spotting locus* mapped to bovine chromosome 6 (BTA6) (Grosz and MacNeil, 1999). In the same chromosome region, Reinsch et al. (1999) and Liu et al.(2009) localised a QTL for the degree of spotting in German Holstein-Friesian and Simmental cattle and in a Holstein-Friesian x Jersey cross. Besides cattle, kit mutation was described as responsible for pigmentation defects in human, mouse and horse (Geissler et al., 1988; Giebel and Spritz, 1991; Haase et al., 2007). According to Haltaufderhide and Oancea (2014), both KIT and TYRO3 are among the 10 highest expressed receptor kinase genes in human epidermal melanocytes.

Durkin et al. (2012) reported that color sidedness in Belgian Blue cattle is determined by a first allele on chromosome 29 (Cs (29)), which results from the translocation of a 492 kb chromosome 6 segment encompassing KIT to chromosome 29. Although, our study were able to find a significant CNV located on chromosome 29, the duplication was found in BTA29:31934125 (first SNP position), which is approximately 10 Mb base pairs upstream from the translocation described by the authors (BTA29:20.8 – 21.2Mb).

The QTL content analysis demonstrated CNVs overlapping a QTL that were previously associated with coat color (QTL ID = 6270) in taurine breeds, which reinforce the potential importance of these region as a candidate. The significant CNvs also overlaps three QTLs involved with heat intensity (QTL IDs = 3569, 5016, 5004) and one QTL involved with heat stress (QTL IDs = 31192). These findings agree with Mader et al. (2002) that demonstrated that coat color had a large impact on the heat tolerance of cattle.

Conclusions

Results suggests that the genomic regions controlling coat color phenotype in both bovine subspecies (taurine and indicine) are similar, and they are associated to coat color in Gyr cattle which are also involved with heat tolerance control.

Table 1. CNVs associated with color coat contrasts in Gyr Breed.

Coat color contrasts	CNV predictor [a]
Chita x Chitado	BovineHD0200033194, BovineHD0300030854, BovineHD0800004068, BovineHD1100031344, BovineHD1200009631, BovineHD1600013398, BovineHD2000000005
Chita x Monochrome	BovineHD0600019880, BovineHD1400008618
Mouro x Monochromes	BTB-00199499, BovineHD0100006355, BovineHD0100018402, BovineHD0100018845, BovineHD0100029600, BovineHD0100047216 BovineHD0200007767, BovineHD0200028760, BovineHD0200038484, BovineHD0300004515, BovineHD0400026873, BovineHD0400032672 BovineHD0500011305, BovineHD0500019746, BovineHD0500021645, BovineHD0600005556, BovineHD0600026173, BovineHD0700019948 BovineHD0700033340, BovineHD0800007537, BovineHD0800011514, BovineHD0800018380, BovineHD0900009034, BovineHD0900020346 BovineHD0900025424, BovineHD1000010927, BovineHD1000019695, BovineHD1100006145, BovineHD1300006924,BovineHD1300006985 BovineHD1400010215, BovineHD1500000585, BovineHD1500013027 BovineHD1500022519, BovineHD1600006751, BovineHD1600016891 BovineHD1700002291, BovineHD1700002359, BovineHD1800005170 BovineHD1800005366, BovineHD1800007727, BovineHD2000005489 BovineHD2000013128, BovineHD2000013512, BovineHD2100017701 BovineHD2200006503, BovineHD2300014492, BovineHD2400012148 BovineHD2700000001, BovineHD2900009553, Hapmap23994-BTA-141224

[a] first SNP from CNV

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