

Identification of pleiotropic key-regulatory genes related to economically relevant traits in beef cattle through systems biology approach

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Summary

In the this investigation, a multi-breed and multi-omics approach was applied to study the pleiotropic effect from a list of key regulatory genes obtained from three independent studies evaluating fertility traits in beef cattle. A pleiotropic map for 32 traits (including growth, feed intake, carcass, meat quality, and reproduction) and a data-mining analysis using the CattleQTLdb were performed to identify candidate genes. This approach allowed the identification of genes with pleiotropic effects related to crucial biological process for fertility, production and health traits such as *IYD*, *RBM20* and *PLA2G4E*. These genes will be further investigated to better understand the biological processes related with these complex traits. These genes could assist in improving selection decisions and reducing the frequency of undesirable genotypes.

Keywords: pleiotropic markers; production and reproduction traits, systems biology, multi-omics, beef cattle.

Introduction

Complex traits are usually regulated by multiple *loci* and several environmental components that have individually a small effects on the phenotypic expression. In addition, *loci* that regulate complex traits generally are involved in several phenotypes, which leads to a pleiotropic effect. However, the real interaction and regulation pattern of these *loci* are poorly understood, specifically in livestock species. Consequently, the use of SNP markers for genomic selection of these traits may result in low accuracy. Additionally, indirect selection

for undesirable traits is common. For example, for both beef and dairy cattle, variants associated with high productive performance can also reduce fertility or affect mortality rates (de Camargo et al., 2015, Tsuruta et al., 2017).

The biological process responsible for sexual maturity, known as puberty, involves complex biological processes which are regulated by several genes that are involved in the development of a wide variety of phenotypes (Dorn and Biro, 2011). Crucial biological structures for puberty development, e.g., pituitary gland, hypothalamus and thyroid, are involved in the regulation of production of several hormones directly related to production traits (Fernández et al., 2017; Nguyen et al., 2017). Furthermore, the deep investigation of the biological processes related to the genes involved with puberty development may result in the identification of key regulatory genes for both fertility and production traits. Within this context, the aim of this study was to integrate multi-omics studies, in a systems biology approach, that investigated pubertal status and fertility traits to identify candidate genes with pleiotropic effects related with economically relevant traits in cattle.

Material and methods

Data collection

In the present research, the genes from three studies were scrutinized. The first study was published by Cánovas et al. (2014) which evaluated genes differentially expressed (DE) between pre- and post- puberty in eight tissues (hypothalamus, pituitary gland, liver, longissimus dorsi muscle, adipose, uterine horn, endometrium, and ovary) from Brangus beef cattle. The second study by Hawken et al. (2012) performed a GWAS for age at puberty, postpartum anestrous interval, and occurrence of the first postpartum ovulation before weaning in the first rebreeding period in Tropical Composite cattle. The third study by Nguyen et al. (2017) reported DE genes in pre- and post- puberty stages for pituitary gland and ovary in Brahman cattle.

Identification of genes nearby pleiotropic markers

The genes identified in the studies described above were mapped against a list of markers with pleiotropic effect ($P < 0.05$ after false discovery rate (FDR) correction) reported by Bolormaa et al. (2014), which analysed 32 traits including growth, feed intake, carcass, meat quality, and reproduction. Using the list of candidate genes obtained by integrating and combining data from omics technologies described by Cánovas et al. (2014), Hawken et al. (2012) and Nguyen et al. (2017), genes up to 1 Mb (downstream and upstream) from a pleiotropic marker were selected.

Genes shared among studies and quantitative trait loci (QTL) mapping

The genes within pleiotropic regions obtained for each study were compared and those in common across the three studies were selected to be mapped against QTL regions using resources from the CattleQTLdb (<https://www.animalgenome.org/cgi-bin/QTLdb/BT/index>). Using an interval of 1 Mb upstream from the start coordinate and 1 Mb downstream from the end coordinate of each one of the shared genes, all the QTLs mapped in this interval were annotated. Those genes mapped in regions where at least five QTL types are annotated (from six possible types: exterior characteristic, health, reproduction, production, meat and carcass

and milk), were ranked as genes with the highest pleiotropic potential.

Results and discussion

Eleven genes were shared among all the three studies (independent populations from three different beef breeds) and mapped in pleiotropic regions (Figure 1). Among them, nine genes were mapped in regions with five or six QTL types annotated. From those nine, three are related to crucial biological processes associated with reproductive and production traits. These genes were: iodotyrosine deiodinase (*IYD*), RNA binding motif protein 20 (*RBM20*) and phospholipase A2 group IVE (*PLA2G4E*). Figure 2 shows the pleiotropic markers mapped around *IYD*, *RBM20* and *PLA2G4E* genes, where it is possible to observe several markers with a significant pleiotropic effect (P-value <0.05 after FDR). These three genes play crucial roles in the regulation of cellular growth, proliferation and differentiation. *IYD* is an enzyme, highly conserved among mammalians, due to its function in the thyroid hormone production. *IYD* is responsible to conserve iodide, during the synthesis of T3 and T4 hormones, from mono- and diiodotyrosine. This process is responsible for maintaining active thyroid hormone production (Moreno and Visser, 2010). *RBM20* is a splicing factor essential to produce titin, a giant polypeptide filament responsible by the sarcomere integrity and assembly (Guo et al., 2012). Titin isoforms transition is regulated by *RBM20* in a process triggered by the thyroid hormone, T3. Knockout mice for *RBM20* under T3 treatment presented an absence of the larger titin isoform (*N2BA*) (Zhu et al., 2015). Changes in titin larger to smaller isoform ratio result in an alteration of the passive tension and force generation of striated muscle (Cazorla et al., 2000). Additionally, in cattle, *RBM20* is mapped to a region with a QTL for Warner-bratzler shear force identified in French beef cattle breeds (Ramayo-Caldas et al., 2016). *PLA2G4E* codify an enzyme responsible for the hydrolyzation of phospholipids into fatty acids. Currently, there are few studies associating the phospholipase A group activity with economically interesting traits, including mastitis resistance, post-partum uterine disease and feed efficiency (Sheldon et al., 2009, Seroussi et al., 2013, Mercadante et al., 2015). Interestingly, thyroid hormones are responsible for inhibition of secretory phospholipase group A (Sharma et al., 2014).

These results indicate a strong influence of thyroid hormones and their pathways over the regulation of multiple biological processes associated with productive traits and developmental processes in cattle. Indeed, changes in the levels of thyroid hormones during pregnancy, mainly in the initial development of the embryos, are associated with adverse pregnancy outcomes and embryonic losses (Twig et al., 2012). Additionally, alterations in thyroid activity may results in male and female infertility (Dittrich et al., 2011, Fernández et al., 2017).

The present study described a multi-breed and multi-omics approach to identify key-regulatory candidate genes for pleiotropic effects in beef cattle using the results generated by previous studies. Further functional analyses will be performed in order to elucidate the relationship between the different traits involved with the pleiotropic effect. Consequently, this study will improve the understanding of the cause and consequence relationships between variants mapped to these genes and complex traits. This systems biology methodology is a powerful tool to identify candidate genes and genomic regions that will aid in the investigation of the underlying biological meaning of economically interesting complex traits in beef cattle.

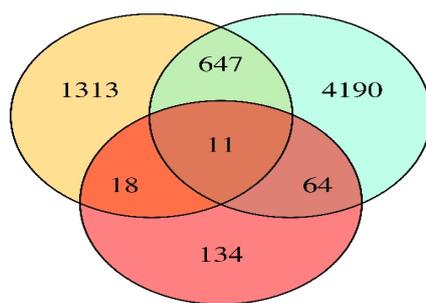


Figure 1. Venn Diagram showing the comparison among the different independent populations analyzed. In blue, the genes identified by Hawken et al., 2012 (Brahman and Tropical Composite breed). In red, the genes identified by Nguyen et al., 2017 (Brahman breed). In yellow, the genes identified by Cánovas et al., 2014 (Brangus breed).

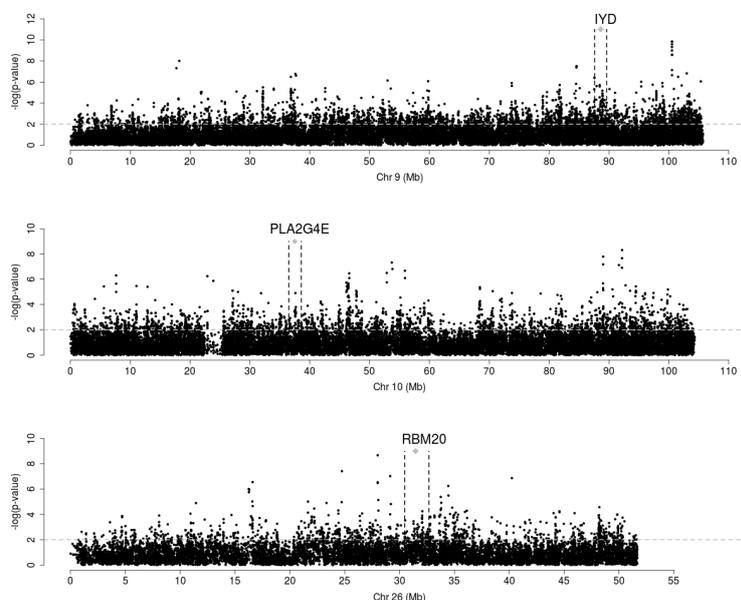


Figure 2. Pleiotropic effect around the genes shared among all studies. The x-axis corresponds to the genomic position in each chromosome and the y-axis to the $-\log(p\text{-value})$. The grey diamond corresponds to the start coordinate of each gene. The vertical dashed lines indicate the 1 Mb interval downstream and upstream used to identify pleiotropic markers close to those genes. The horizontal dashed lines indicate the 5% genome-wide FDR threshold.

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