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EPIGENETIC REPRESSION OF GENES ASSOCIATED WITH RIBEYE AREA OF NELORE CATTLE

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SUMMARY

Understanding the epigenetic repression role in regulating genes involved with the ribeye area (REA) of bovine muscle can help us to predict this trait in the future. Here, we identified genes putatively regulating REA in Nelore cattle and divergently epigenetically repressed between contrasting sample groups. For that, we applied the TRIAGE method with a Rank Product analysis using bovine muscle expression data on high versus low REA groups. Further, we identified over-represented pathways and biological processes linked to candidate genes, searching for their regulatory direction. This result advances the knowledge about how epigenetic regulation may impact production traits in Nelore cattle.

INTRODUCTION

The ribeye area (REA) of the bovine *Longissimus dorsi* muscle is used as an indirect measure of carcass composition (Miar *et al*. 2013). The complete regulation of this trait is not known. As such, identifying candidate genes modulating REA is important. Additionally, delineating the mechanisms underlying the modulation of candidate genes would lead to a better understanding of this complex trait. Based on that, we aimed to identify genes regulating REA and that are also being putatively epigenetically repressed in one of the contrasting sample groups for REA. The lack of data on epigenetic repression mechanisms linked to bovine muscle tissue is a limitation. However, our approach can predict genes discordantly activated by epigenetic repression mechanisms considering only expression data. This methodology, named TRIAGE, consists of a repressive tendency score calculated for human genes. We applied this score to the expression value for each gene, in each sample, to calculate a bovine discordant score that can predict genes being affected by repressive mechanisms in each sample (Shim *et al*. 2020). TRIAGE was then expanded using a Rank Product analysis (Afonso *et al*. 2023) to allow us to compare discordant scores between the REA contrasting groups.

MATERIALS AND METHODS

Samples, phenotypes and expression. The genetic estimated breeding values (GEBV) for Ribeye area (REA) from contrasting Nelore steers groups and their *Longissimus thoracis* muscle's expression data from an RNA-Seq experiment were previously described by Silva-Vignato *et al.* (2017). In short, we used the RNA-Seq data of 12 Nelore steers muscle samples representing contrasting GEBV groups for REA. These 12 animals were selected out of 385 samples from a research population from Embrapa (Brazilian Agricultural Research Corporation, São Paulo, SP, Brazil), representing the Brazilian breeding lineages in 2009.

DRGs identification. We implemented a combination of the TRIAGE method (Shim *et al*. 2020) with the RankProd R package (Hong *et al*. 2006) using the expression data to identify putatively epigenetic repressed genes affecting the REA trait, called herein discordantly regulated genes (DRGs). The TRIAGE method is based on the inverse relationship between H3K27me3 histone modification and human gene expression and can be extrapolated to any species (Shim *et al*. 2020). The outputs of this analysis are ranks of genes per sample regarding their discordant score (DS). These DS represent the discordance between the expected expression and the real one based on the chance of this gene being epigenetically repressed. These DS were compared between the contrasting groups with the RankProd R package to identify the DRGs.

Putative relationship between DRGs and REA. In search of the link between the DRGs and REA, we used the PCIT algorithm (Reverter *et al.* 2008) and the Cytoscape software (Shannon *et al.* 2003) to construct a correlation network. The correlation analysis with PCIT was made with all the expression data and REA GEBV for all 12 samples. The correlated pairs containing at least one DRG or the REA GEBV were considered for the network analysis. The genes significantly correlated with each DRGs were used in separate functional annotation analysis with the STRING software (Pertea *et al.* 2015) to retrieve GO terms and metabolic pathways from known protein-protein interaction, considering the product proteins of the DRGs. Subsequently, different sources of information were used to characterize genes present in the network: 1) enriched terms from our functional annotation analysis; 2) previously published differentially expressed genes (Silva-Vignato *et al.* 2017); 3) bovine transcription factors (de Souza *et al.* 2018); 4) bovine known miRNAs. Thus, we identified putative regulatory processes by the functional annotation analysis and other known regulatory (miRNA or TF) or REA related genes (DEG or correlated to REA), depending on their attributes and their correlation with REA or a DRG.

RESULTS AND DISCUSSION

DRGs for REA. We identified six DRGs for REA. The DRGs are the candidate regulators for REA that are also putatively being affected by a repressive epigenetic mechanism. They were differentially ranked between contrasting groups by our choose method because they have a high tendency to be repressed in several tissues but presented an expression between contrasting groups for REA. This is an indicator of epigenetically repression. One DRG was significant in the comparison considering High REA x Low REA and five DRGs were significant in the comparison considering Low REA x High REA (pfp < 0.01). The difference in expression between both contrasting groups shows that the only DRG for the comparison High REA x Low REA (*CDH22*) presented higher expression in the Low REA group. Based on the methodology assumption, this can be interpreted as an indication of epigenetic repression of this DRG in the High REA group. The same is valid on the contrary for the other five DRGs, being DRGs for the comparison Low REA x High REA and presenting a higher expression in the High REA group. Figure 1 shows the DRGs, the percentage of false positive (pfp) indicating its significance in the analysis (A) and their expression differences between the groups (B). DRGs can affect the trait in the study by regulating biological processes, while being epigenetically repressed by H3K27me3 or other epigenetic

repressive mechanisms (Afonso *et al*. 2023), proposing a new layer of understanding regarding the biological regulation linked to REA.

Putative relationship between DRGs and REA. Figure 2 presents the correlation network considering the significant correlations containing at least one DRG or REA, and its attributes pointing to regulatory functions (miRNAs, TFs and DRGs) and its known relationship with REA (previously published Differentially expressed genes, DEGs, for REA, Silva-Vignato *et al*. 2017). No DRG for REA was previously published as DEG for REA (Silva-Vignato *et al*. 2017).

A)

DRGs	high_up_pfp	low_up_pfp
CDH22	**4.11E-04**	9.62E-01
LBX1	1.22E+00	**2.01E-03**
ZIC4	1.19E+00	**6.18E-03**
CNTFR	9.53E-01	**7.94E-03**
COL2A1	9.12E-01	**9.17E-03**
EN1	9.27E-01	**9.43E-03**

B)

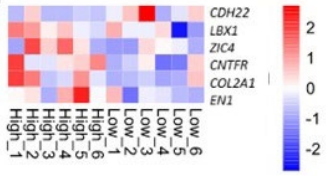

Figure 1. Discordantly regulated genes (DRGs) for Ribeye area (REA) in Nelore

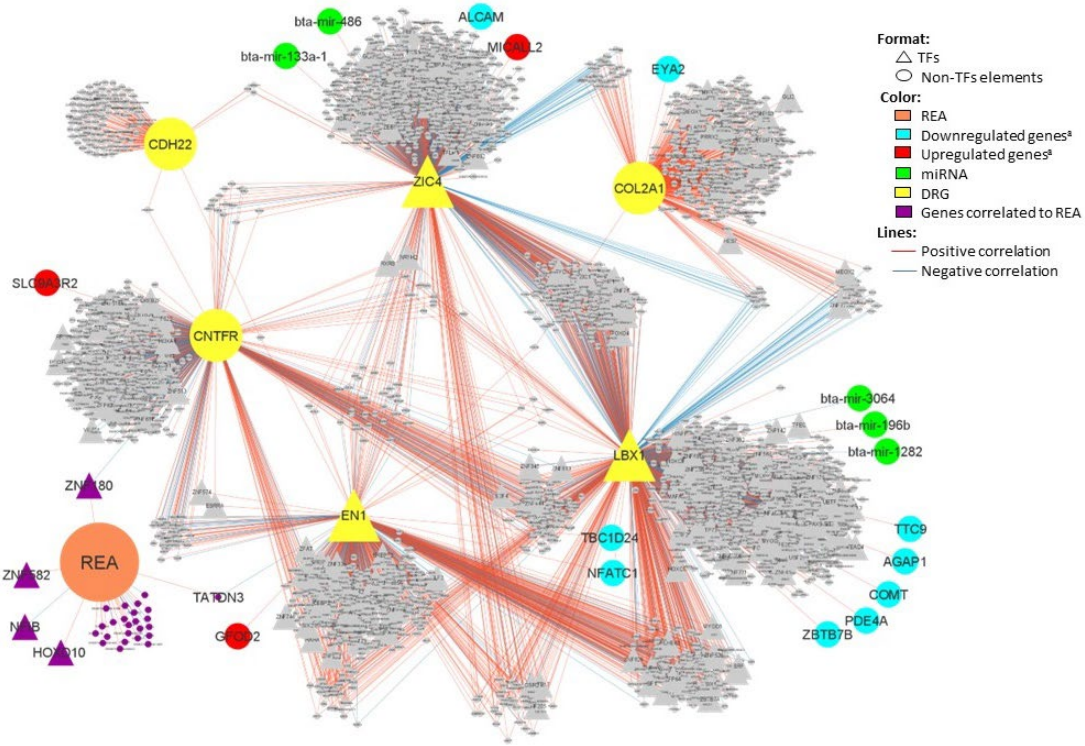

Figure 2. Correlation network focused on the first neighbours of Discordantly Regulated Genes (DRGs) and Ribeye Area (REA) in Nelore [a]Genes downregulated or upregulated in the Low REA group when compared to the High REA group.

All five DRGs for the comparison Low REA x High REA are correlated to at least one DEG for REA, showing its previously known link to REA. Three of the six DRGs are TFs *(ZIC4, LBX1* and *EN1*), and two of these TFs are correlated to miRNAs (*ZIC4* and *LBX1*), indicating its regulatory nature. Considering our network (Figure 2), the expression of the DRGs is not directly correlated to

the REA GEBV, but there are two genes directly correlated to REA that are correlated to two different DRGs (the TF *ZNF180* correlated to the DRG *CNTFR* and *TATDN3* correlated to the DRG and TF *EN1*), which are also candidate regulators to the REA, with all the DRGs.

The DRG correlated to more DEGs for REA (Silva-Vignato *et al*. 2017) is *LBX1*, a TF also correlated to genes enriched for the two pathways enriched for the DEGs related to REA (Silva-Vignato *et al*. 2017): MAPK signalling and endocytosis pathways. Considering all the results from the functional annotation analysis, we noted that the *ZIC4* and *CDH22* DRGs were mainly correlated to genes enriched for pathways and processes related to immunity and metabolism. The *CNTFR* gene was involved with protein and DNA regulation, *EN1* to histone modification, protein transport and chromatin regulations, *LBX1* to protein, transcription, DNA-template, growth and cell death regulations, and *COL2A1* to an extracellular matrix organization, synthesis and degradation and protein digestion and absorption. All these pathways and processes can be related to muscle growth, organization, degradation and fat deposition, which are key biological process to REA (Silva-Vignato *et al*. 2017).

CONCLUSIONS

Our approach helped us to point to candidate regulatory genes for REA, also being putatively epigenetically regulated. Also, we identified the possible pathways and biological processes being regulated by each DRG and other candidate regulatory genes underlying REA.

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