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Research article

Transcriptome profiling reveals differential expression of genes potentially involved in muscle and adipose tissue development of cattle

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ABSTRACT

Background: To identify differentially expressed genes (DEGs) between muscle and adipose in cattle, we analyzed the data from the RNA sequencing of three Angus × Qinchuan crossbred cattle.
Results: Searched the Gene Expression Omnibus (GEO) for a microarray dataset of Yan yellow cattle, GSE49992. After the DEGs were identified, we used STRING and Cytoscape to construct a protein–protein interaction (PPI) network, subsequently analyzing the major modules of key genes. In total, 340 DEGs were discovered, including 21 hub genes, which were mainly enriched in muscle contraction, skeletal muscle contraction, troponin complex, lipid particle, Z disc, tropomyosin binding, and actin filament binding.
Conclusions: In summary, these genes can be regarded as candidate biomarkers for the regulation of muscle and adipose development.
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1. Introduction

The quality of meat is affected by a higher fat content [1], which has an important impact on the biological composition and affects the juiciness and flavor. Currently, consumers are paying more attention to their health and demanding better tasting and healthier meat. Fat is made up of large clusters of adipocytes and primarily functions in animals as energy storage and as an endocrine organ. Fat is of importance in the regulation of metabolism and fat deposition, and it can improve animal health and meat quality [2,3].

Adipocytes mainly gather under the subcutaneous, intermuscular, visceral, and mesentery connective tissue, and some are scattered between and inside the muscle bundle; while on one hand, intramuscular or marbling fat plays a vital role in improving flavor and palatability of meat, on the other, subcutaneous and visceral fat pads are considered to be of no worth [4]. When subjected to texture profile analysis (TPA), compared with the high-fat control, the chewability of the low-fat sample increased, while the hardness and elasticity remained unchanged [5]. In many cases, muscle and fat selection are product-driven. Previous research has shown that after adjusting for intramuscular fat, Wagyu had more intense flavor and higher tenderness and juiciness compared to Angus [6]; for example, at the level of gene regulation, *KLF3* gene [7] and *SIRT1* gene [8] alter intramuscular fat content in cattle. Muscle and adipose functionality are affected by a comprehensive genetic regulatory network. Biological explanations, especially for DEGs, remain a challenge with RNA-seq. Further research is required to discover the genes and their mechanisms which make a difference in bovine muscle and fat function, particularly through mining hub genes (biomarkers). In this study, integration with functional enrichment, pathway analysis, and a protein–protein interaction (PPI) network was used to analyze DEGs between the longissimus dorsi muscle and adipose tissues.

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2. Materials and methods

2.1. Experimental cattle

The cattle for this experiment came from the breeding ground of the National Beef Cattle Improvement Center (Yangling, China), with the same feeding conditions as supported by the ethics committee of the Institutional Animal Care and Use Committee (Northwest A&F University, China).

Three 18-month-old Angus × Qinchuan crossbred cattle were slaughtered, and the longissimus dorsi and subcutaneous adipose tissues over the 12th to 13th rib over the midline of the carcass were sampled and stored at −80°C. The experiment was conducted on the longissimus dorsi muscle tissue and subcutaneous adipose tissue group, with the three replicate animals for each tissue identified using the following code: RAN-Q-M102 RAN-Q-M12, RAN-Q-M89, and RAN-Q-F102, RAN-Q-F12, RAN-Q-F89.

2.2. RNA extraction, sequencing, and mapping

For the procedure of extraction of total RNA and sequencing, we referred to Mei et al. [9]. Then, after using the FASTX-Toolkit [10] to control the quality of raw reads and removing the reads containing the adapter, reads containing over 10% poly-Ns, and reads of low quality (>50% of bases with Phred scores <10), we mapped the extracted sequence to the reference genome (UMD3.1) of cattle with TopHat v2.0.9 [11] and Bowtie v2.0.6 [12] using the default parameters.

2.3. Microarray data

One dataset [GSE49992] (GPL2112 platform, Affymetrix Bovine Genome Array) was downloaded from the Gene Expression Omnibus (GEO) [13], where a total of nine bulls of the same breed (Yan yellow cattle) were included, and the dataset contained three longissimus dorsi muscle tissues samples, three subcutaneous adipose tissues samples, and three abdominal adipose tissues samples.

2.4. Identification of DEGs

The DESeq (1.18.0) [14] was used for analyzing DEGs from the transcriptome data of crossbred cattle. Meanwhile, the DEGs between muscle tissue and adipose tissue samples downloaded from GEO datasets were analyzed using GEO2R, an interactive web tool, with $|\log FC| > 2$ and adj. P-value < 0.001 as the criterion of significance. Downloaded data from GEO were divided into three groups: the LodAba group, for the longissimus dorsi muscle and abdominal adipose tissues; the LodSua group, for the longissimus dorsi muscle and subcutaneous adipose tissues; and the LodAdi group, for the longissimus dorsi muscle and adipose tissues.

2.5. GO enrichment analyses and KEGG of DEGs

We used the DAVID online database (version 6.8; http://david.ncifcrf.gov) [15] to analyze the function, biological processes, and KEGG of DEGs to provide comprehensive information regarding the gene and protein functions. To analyze the DEGs, FDR <0.05 was considered as the criterion of significance.

2.6. PPI network and module analyses

For the DEGs, we used the STRING online database [16] to predict the PPI network, with an interaction score > 0.4. Then, we used Cytoscape (version 3.7.1) [17] and Molecular Complex Detection (MCODE) (version 1.5.1) [18] to cluster key modules from the PPI network by the default parameters. Subsequently, the functional major module genes were analyzed using DAVID. Meanwhile, the R visualization package GOPlot [19] was used to obtain a visualization of the relationships between genes and the functional categories.

3. Results

3.1. RNA-Seq data analyses

We obtained a total of 260.9 million clean reads, with approximately 88.71% of these reads aligned to the reference genome, of which 70.07% were unique (Table 1).

3.2. Discovered DEGs in muscle tissue and adipose tissue

Through standardization of the results, the DEGs (889 in LodAba group, 912 in LodSua group, 957 LodAdi group from Yan yellow cattle, and 1832 in AnQin group from Angus×Qinchuan crossbred cattle) were confirmed, along with the overlap of four groups, including 340 genes displayed in a Venn diagram (Fig. 1A).

3.3. Enrichment and KEGG analyses of DEGs

For the gene ontology (GO) term analyses of biological processes (BP), DEGs were significantly clustered in skeletal muscle contraction (GO:0003009), myofibril assembly (GO:0030239), gluconeogenesis (GO:0006094), and muscle contraction (GO:0006936) (Table 2). Cell components (CC) of DEGs were significantly clustered in the Z disc (GO:0030018), extracellular exosome (GO:0070062), troponin complex (GO:0005861), and blood microparticle (GO:0072562) (Table 2). Molecular functions (MF) were clustered and enriched in actin filament binding (GO:0051015), actin binding (GO:0003779), calcium ion binding (GO:0005509), and FATZ binding (GO:0051373) (Table 2). KEGG pathway analyses showed that DEGs were greatly enriched in adrenergic signaling in cardiomyocytes (bta04261), pertussis (bta05133), cardiac muscle contraction (bta04260), and carbon metabolism (bta01200) (Table 2).

3.4. PPI network and module analyses

The PPI network of DEGs was produced using STRING (Fig. 1B), and core modules were generated using Cytoscape (Fig. 1C–E). Functional enrichment analyses of these modules' genes were mainly enriched in muscle contraction (GO:0006936), skeletal muscle contraction (GO:0003009), troponin complex (GO:0005861), lipid particle (GO:0005811), Z disc (GO:0030018), tropomyosin binding (GO:0005523), and actin filament binding (GO:0051015) (Table 3, Fig. 2).

Table 1
The results of the six samples aligned to the reference genome.

Sample	Clean reads	Total mapped (%)	Uniquely mapped (%)
RAN-Q-M102	42,087,136	87.27%	67.01%
RAN-Q-M12	27,164,290	86.46%	67.07%
RAN-Q-M89	49,965,854	87.57%	66.96%
RAN-Q-F102	47,120,010	91.53%	73.74%
RAN-Q-F12	47,828,122	89.62%	72.91%
RAN-Q-F89	46,766,436	89.83%	72.75%
Total/Average	260,931,848	88.71%	70.07%

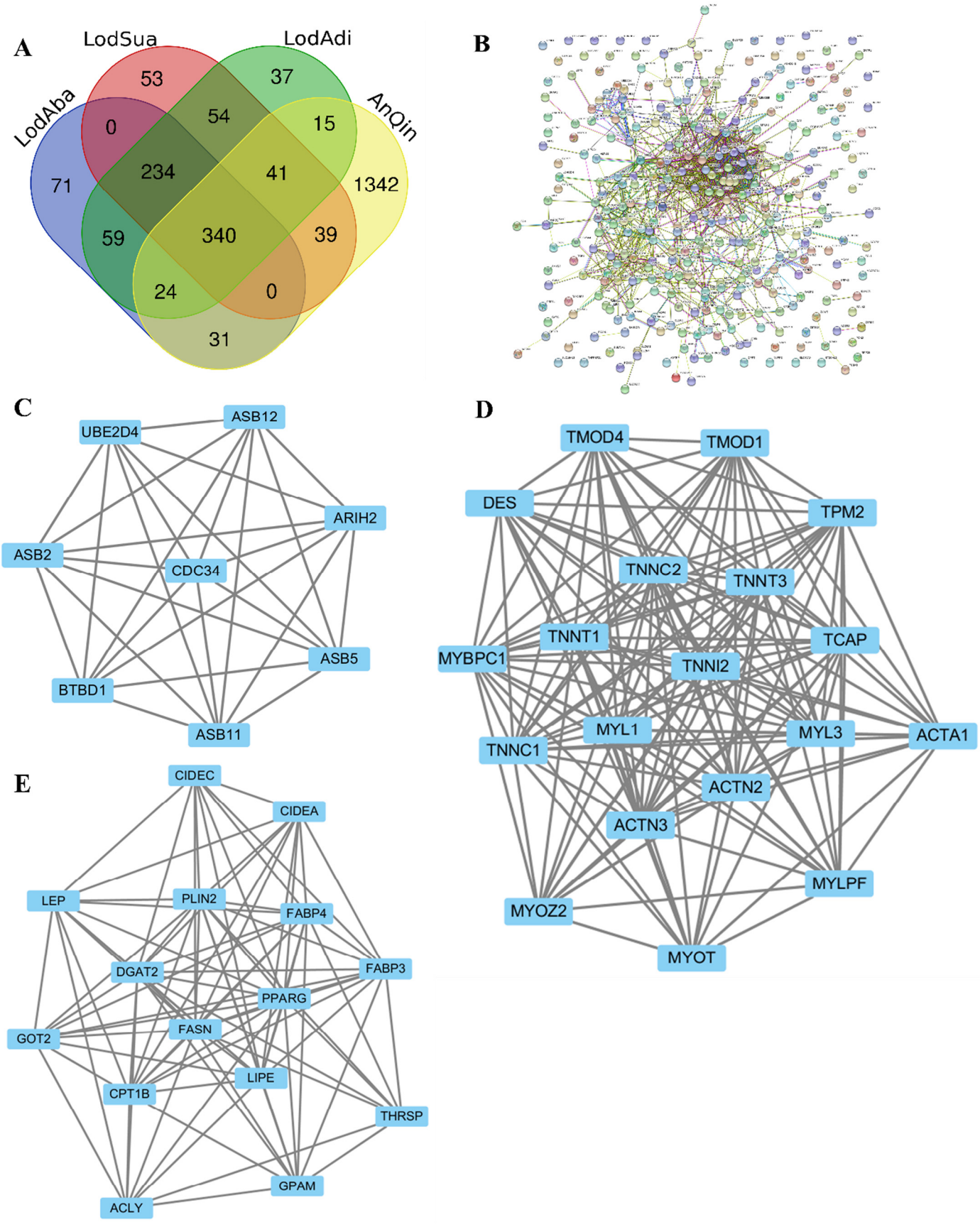

Fig. 1. Venn diagram of the protein–protein interaction (PPI) network and the most significant module of differentially expressed genes (DEGs). (A) The DEGs were selected with a fold change >2 and adj. *P*-value < 0.001 among the four groups: the LodAba group, for the longissimus dorsi muscle and abdominal adipose tissues; the LodSua group, for the longissimus dorsi muscle and subcutaneous adipose tissues; the LodAdi group, for the longissimus dorsi muscle and adipose tissues; and the AnQin group, for the Angus × Qinchuan crossbred cattle longissimus dorsi muscle and subcutaneous adipose tissues. (B) The PPI network of DEGs was constructed by STRING. (C-E) Molecular Complex Detection (MCODE) modules from DEG screening.

Table 2
Gene ontology (GO) terms and KEGG pathway analyses of DEGs.

Category	Term	Description	Count in gene set	FDR
Biological processes (BP)	GO:0003009	Skeletal muscle contraction	7	0.00119
	GO:0030239	Myofibril assembly	6	0.00134
	GO:0006094	Gluconeogenesis	7	0.00487
	GO:0006936	Muscle contraction	8	0.00633
Cell components (CC)	GO:0030018	Z disc	19	5.22E-12
	GO:0070062	Extracellular exosome	85	3.17E-07
	GO:0005861	Troponin complex	5	0.0122
	GO:0072562	Blood microparticle	10	0.0199
Molecular functions (MF)	GO:0051015	Actin filament binding	12	5.10E-05
	GO:0003779	Actin binding	12	0.00300
	GO:0005509	Calcium ion binding	29	0.0234
	GO:0051373	FATZ binding	4	0.0469
KEGG	bta04261	Adrenergic signaling in cardiomyocytes	16	3.63E-04
	bta05133	Pertussis	11	0.00863
	bta04260	Cardiac muscle contraction	11	0.0122
	bta01200	Carbon metabolism	12	0.0347

4. Discussion

In this study, we demonstrated that many genes have an important influence on muscle and adipose functionality. Genes of actinin alpha 2 (*ACTN2*) and actinin alpha 3 (*ACTN3*) constitute the Z-line in mammalian skeletal muscle fibers and can be altered by endoplasmic reticulum (ER) stress, which may modulate muscle performance [20]. Myosin-binding protein C, slow type (*MYBPC1*), has been used to increase beef productivity [21]. Myosin light chain 1 (*MYL1*); troponin T1, slow skeletal type (*TNNT1*); and troponin T3, fast skeletal type (*TNNT3*), affect the tenderness of meat through oxidation and act on the proteins of different metabolic pathways [22]. Titin-cap (telethonin) (*TCAP*), one of the components of the z-disk, regulates muscle growth and development, which is exclusively expressed in muscle tissue [23]. Tropomodulin 1 (*TMOD1*) and tropomodulin 4 (*TMOD4*) are part of the TMOD family and have an important impact on thin-filament length regulation [24], whereas TMOD4 was required for thin-filament length regulation [25,26]. In particular, TMOD1 was found to be a novel regulatory factor for skeletal muscle physiology [27]. Troponin C1, slow skeletal and cardiac type (*TNNC1*), is proposed to be related with tenderness [28]. Troponin C2, fast skeletal type (*TNNC2*), was found in the regions associated with longissimus muscle area (LMA), which implies that it can strongly be related with fat deposition [29]. Troponin I2, fast skeletal type (*TNNI2*), and tropomyosin 2 (beta) (*TPM2*) regulate muscle processes of contraction and skeletal muscle structure [30]. Furthermore, myozenin 2 (*MYOZ2*) can regulate the differentiation of myoblasts [31], by Myocyte enhancer factor 2A (MEF2A) which was an important transcription factor. With fat tissue as an active endocrine organ, the endocrine system and tissue growth regulation of the body's processes have become research hot spots [32]. The genes of cell-death-inducing DFFA-like effector a (*CIDEA*) and cell-death-inducing DFFA-like effector c (*CIDEC*) have been considered as important regulators of lipid homeostasis [33]. Diacylglycerol O-acyltransferase 2 (*DGAT2*) can reduce diet-induced hepatic steatosis (HS) with a low level [34]. Hormone-sensitive lipase (*LIPE*) is an intracellular neutral lipase [35] which relates to oleic acid and total monounsaturated fatty acids metabolism. Perilipin 2 (*PLIN2*) are associated with maintenance of adipose cells and their components. Li et al. [36] revealed the mechanism through which PLIN2 regulates lipid droplets during the early formation and accumulation of intracellular lipid content [37].

In conclusion, this study showed that the following results: (1) a total of 340 DEGs, which included 21 hub genes, were identified, and the hub genes can be regarded as candidate biomarkers for muscle and adipose functionality; and (2) these hub genes revealed their functional roles in lipid metabolism which may be useful for modifying sensory attributes which are associated with fat in the meat such as the flavor and texture. Due to the lack of other studies in this area, the mechanisms responsible for these effects remain unknown and require further research. These results should help to better understand the genetic and physiological mechanisms that regulate muscle tissue and subcutaneous fat expression and might be useful for cattle breeding.

Conflicts of interest

The authors declare no conflict of interest.

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Table 3
GO term enrichment analysis of significant module genes.

Category	Term	Description	Genes	Count	FDR
BP	GO:0006936	Muscle contraction	MYBPC1, MYL1, TMOD4, ACTN2, ACTN3, TPM2, TMOD1	7	6.31E-07
	GO:0003009	Skeletal muscle contraction	TNNT3, TNNT1, TCAP, TNNC2, TNNC1, TNNI2	6	1.40E-06
CC	GO:0005861	Troponin complex	TNNT3, TNNT1, TNNC2, TNNC1, TNNI2	5	2.94E-06
	GO:0005811	Lipid particle	PLIN2, DGAT2, CIDEA, LIPE, CIDEC	6	1.77E-04
	GO:0030018	Z disc	DES, TCAP, ACTN2, ACTN3, MYOZ2, MYOT	6	0.001527
MF	GO:0005523	Tropomyosin binding	TNNT3, TNNT1, TMOD4, TMOD1	4	0.001417
	GO:0051015	Actin filament binding	TNNC2, TNNC1, TMOD4, TPM2, TMOD1	5	0.018969

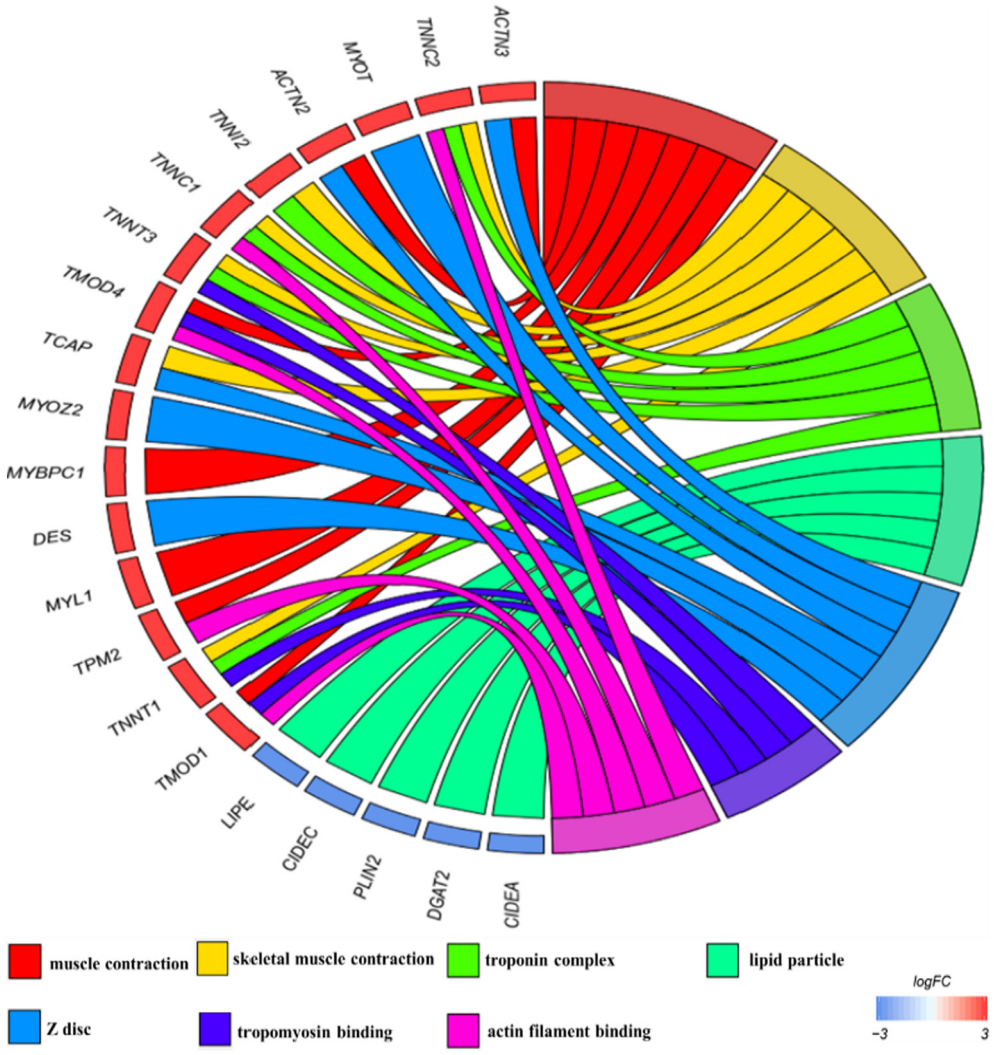

Fig. 2. Distribution of genes from the GO analyses of DEGs in major modules.

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Supplementary material

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References

[1] Ventanas S, Ventanas J, Jurado Á, et al. Quality traits in muscle *biceps femoris* and back-fat from purebred Iberian and reciprocal Iberian×Duroc crossbred pigs. Meat Sci. 2006;73:651–9. https://doi.org/10.1016/j.meatsci.2006.03.009 PMid: 22062566.

[2] Alessi MC, Peiretti F, Morange P, et al. Production of plasminogen activator inhibitor 1 by human adipose tissue: Possible link between visceral fat accumulation and vascular disease. Diabetes. 1997;46:860–7. PMid: 9133556.

[3] Avram MM, Avram AS, James WD. Subcutaneous fat in normal and diseased states. J Am Acad Dermatol. 2007;56:472–92. https://doi.org/10.1016/j.jaad.2006.06.022 PMid: 17317490.

[4] Li X, Fu X, Yang G, et al. Review: Enhancing intramuscular fat development via targeting fibro-adipogenic progenitor cells in meat animals. animal. 2019;14:312–21. https://doi.org/10.1017/S175173111900209X PMid: 31581971.

[5] El-Magoli SB, Laroia S, Hansen PM. Flavor and texture characteristics of low fat ground beef patties formulated with whey protein concentrate. Meat Sci. 1996;42:179–93. https://doi.org/10.1016/0309-1740(95)00032-1.

[6] Frank D, Ball A, Hughes J, et al. Sensory and flavor chemistry characteristics of Australian beef: Influence of intramuscular fat, feed, and breed. J Agric Food Chem. 2016;64:4299–311. https://doi.org/10.1021/acs.jafc.6b00160 PMid: 27118482.

[7] Guo H, Raza SHA, Schreurs NM, et al. Genetic variants in the promoter region of the KLF3 gene associated with fat deposition in Qinchuan cattle. Gene. 2018;672:50–5. https://doi.org/10.1016/j.gene.2018.06.022 PMid: 29890309.

[8] Gui L-S, Raza SHA, Sun Y-G, et al. Detection of polymorphisms in the promoter of bovine SIRT1 gene and their effects on intramuscular fat content in Chinese indigenous cattle. Gene. 2019;700:47–51. https://doi.org/10.1016/j.gene.2019.03.022 PMid: 30902782.

[9] Mei C, Li S, Abbas SH, et al. Performance measurement and comparative transcriptome analysis revealed the efforts on hybrid improvement of Qinchuan cattle. Anim Biotechnol. 2019;30:13–20. https://doi.org/10.1080/10495398.2017.1420662 PMid: 29402192.

[10] Schmieder R, Edwards R. Quality control and preprocessing of metagenomic datasets. Bioinformatics (Oxford, England). 2011;27:863–4. https://doi.org/10.1093/bioinformatics/btr026 PMid: 21278185.

[11] Kim D, Pertea G, Trapnell C, et al. TopHat2: Accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 2013;14:R36. https://doi.org/10.1186/gb-2013-14-4-r36 PMid: 23618408.

[12] Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9. https://doi.org/10.1038/nmeth.1923 PMid: 22388286.

[13] Edgar R, Domrachev M, Lash AE. Gene expression omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 2002;30:207–10. https://doi.org/10.1093/nar/30.1.207 PMid: 11752295.

[14] Anders S, Huber W. Differential expression analysis for sequence count data. Genome Biol. 2010;11:R106. https://doi.org/10.1186/gb-2010-11-10-r106 PMid: 20979621.

[15] Huang DW, Sherman BT, Tan Q, et al. The DAVID gene functional classification tool: A novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol. 2007;8:R183. https://doi.org/10.1186/gb-2007-8-9-r183 PMid: 17784955.

[16] Franceschini A, Szklarczyk D, Frankild S, et al. STRING v9.1: Protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2013;41: D808–15. https://doi.org/10.1093/nar/gks1094 PMid: 23203871.

[17] Shannon P, Markiel A, Ozier O, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13: 2498–504. https://doi.org/10.1101/gr.1239303 PMid: 14597658.

[18] Bandettini WP, Kellman P, Mancini C, et al. MultiContrast Delayed Enhancement (MCODE) improves detection of subendocardial myocardial infarction by late gadolinium enhancement cardiovascular magnetic resonance: A clinical validation study. J Cardiovasc Magn Reson. 2012;14:83. https://doi.org/10.1186/1532-429X-14-83 PMid: 23199362.

[19] Walter W, Sánchez-Cabo F, Ricote M. GOplot: An R package for visually combining expression data with functional analysis. Bioinformatics (Oxford, England). 2015; 31:2912–4. https://doi.org/10.1093/bioinformatics/btv300 PMid: 25964631.

[20] Harada N, Gotoda Y, Hatakeyama A, et al. Differential regulation of *Actn2* and *Actn3* expression during unfolded protein response in C_2C_{12} myotubes. J Muscle Res Cell Motil. 2020. https://doi.org/10.1007/s10974-020-09582-7 PMid: 32451822.

[21] Tong B, Xing YP, Muramatsu Y, et al. Association of expression levels in skeletal muscle and a SNP in the MYBPC1 gene with growth-related trait in Japanese black beef cattle. J Genet. 2015;94:135–7. https://doi.org/10.1007/s12041-015-0471-7 PMid: 25846887.

[22] Malheiros JM, Braga CP, Grove RA, et al. Influence of oxidative damage to proteins on meat tenderness using a proteomics approach. Meat Sci. 2019;148:64–71. https://doi.org/10.1016/j.meatsci.2018.08.016 PMid: 30317011.

[23] He H, Hu ZG, Tserennadmid S, et al. Novel muscle-specific genes TCAP, TNNI1, and FHL1 in cattle: SNVs, linkage disequilibrium, combined genotypes, association analysis of growth performance, and carcass quality traits and expression studies. Anim Biotechnol. 2018;29:259–68. https://doi.org/10.1080/10495398.2017.1377084 PMid: 29095095.

[24] Zhao X, Huang Z, Liu X, et al. The switch role of the *Tmod4* in the regulation of balanced development between myogenesis and adipogenesis. Gene. 2013;532: 263–71. https://doi.org/10.1016/j.gene.2013.08.088.

[25] Berger J, Tarakci H, Berger S, et al. Loss of Tropomodulin4 in the zebrafish mutant *trage* causes cytoplasmic rod formation and muscle weakness reminiscent of nemaline myopathy. Dis Model Mech. 2014;7:1407–15. https://doi.org/10.1242/dmm.017376 PMid: 25288681.

[26] Gokhin DS, Tierney MT, Sui Z, et al. Calpain-mediated proteolysis of tropomodulin isoforms leads to thin filament elongation in dystrophic skeletal muscle. Mol Biol Cell. 2014;25:852–65. https://doi.org/10.1091/mbc.e13-10-0608 PMid: 24430868.

[27] Gokhin DS, Lewis RA, McKeown CR, et al. Tropomodulin isoforms regulate thin filament pointed-end capping and skeletal muscle physiology. J Cell Biol. 2010;189: 95–109. https://doi.org/10.1083/jcb.201001125 PMid: 20368620.

[28] Boudon S, Ounaissi D, Viala D, et al. Label free shotgun proteomics for the identification of protein biomarkers for beef tenderness in muscle and plasma of heifers. J Proteomics. 2020;217:103685. https://doi.org/10.1016/j.jprot.2020.103685 PMid: 32058039.

[29] Silva RP, Berton MP, Grigoletto L, et al. Genomic regions and enrichment analyses associated with carcass composition indicator traits in Nellore cattle. J Anim Breed Genet. 2019;136:118–33. https://doi.org/10.1111/jbg.12373 PMid: 30592105.

[30] Ochala J. Thin filament proteins mutations associated with skeletal myopathies: Defective regulation of muscle contraction. J Mol Med (Berl). 2008;86:1197–204. https://doi.org/10.1007/s00109-008-0380-9 PMid: 18574571.

[31] Wang YN, Yang WC, Li PW, et al. Myocyte enhancer factor 2A promotes proliferation and its inhibition attenuates myogenic differentiation via myozenin 2 in bovine skeletal muscle myoblast. PLoS One. 2018;13:e0196255. https://doi.org/10.1371/journal.pone.0196255 PMid: 29698438.

[32] Schutte AE, van Vuuren D, van Rooyen JM, et al. Inflammation, obesity and cardiovascular function in African and Caucasian women from South Africa: The POWIRS study. J Hum Hypertens. 2006;20:850–9. https://doi.org/10.1038/sj.jhh.1002065 PMid: 16855625.

[33] Chen FJ, Yin Y, Chua BT, et al. CIDE family proteins control lipid homeostasis and the development of metabolic diseases. Traffic (Copenhagen, Denmark). 2019;21: 94–105. https://doi.org/10.1111/tra.12717 PMid: 31746121.

[34] Gluchowski NL, Gabriel KR, Chitraju C, et al. Hepatocyte deletion of triglyceride-synthesis enzyme acyl CoA: Diacylglycerol acyltransferase 2 reduces steatosis without increasing inflammation or fibrosis in mice. Hepatology (Baltimore, Md). 2019;70: 1972–85. https://doi.org/10.1002/hep.30765 PMid: 31081165.

[35] Goszczynski DE, Mazzucco JP, Ripoli MV, et al. Characterization of the bovine gene LIPE and possible influence on fatty acid composition of meat. Meta Gene. 2014;2: 746–60. https://doi.org/10.1016/j.mgene.2014.09.001 PMid: 25606458.

[36] Li P, Wang Y, Zhang L, et al. The expression pattern of PLIN2 in differentiated adipocytes from Qinchuan cattle analysis of its protein structure and interaction with CGI-58. Int J Mol Sci. 2018;19:1336. https://doi.org/10.3390/ijms19051336 PMid: 29723991.

[37] Conte M, Armani A, Conte G, et al. Muscle-specific Perilipin2 down-regulation affects lipid metabolism and induces myofiber hypertrophy. J Cachexia Sarcopenia Muscle. 2019;10:95–110. https://doi.org/10.1002/jcsm.12355 PMid: 30288961.