https://doi.org/10.17221/103/2022-CJAS

Differentially expressed genes in the *longissimus dorsi* muscle between the Chinese indigenous Ningxiang pig and Large White breed using RNA sequencing

Fang Wang[1], Linfeng Lei[1,2], Zhaobin Wang[2], Yulong Yin[1], Huansheng Yang[2], Zhe Yang[1], Jiashun Chen[1]**

[1]*Animal Nutritional Genome and Germplasm Innovation Research Center, College of Animal Science and Technology, Hunan Agricultural University, Changsha, P.R. China*
[2]*Hunan International Joint Laboratory of Animal Intestinal Ecology and Health, Laboratory of Animal Nutrition and Human Health, College of Life Sciences, Hunan Normal University, Changsha, P.R. China*

**Corresponding authors: zheyang@hunau.edu.cn; jschen@hunau.edu.cn*

Citation: Wang F., Lei L.F., Wang Z.B., Yin Y.L., Yang H.S., Yang Z., Chen J.S. (2022): Differentially expressed genes in the *longissimus dorsi* muscle between the Chinese indigenous Ningxiang pig and Large White breed using RNA sequencing. Czech J. Anim. Sci., 67: 442–453.

Abstract: High intramuscular fat content of pigs improves pork quality and increasing intramuscular fat deposition is a long-term goal in the husbandry of pigs reared for meat production. There are significant phenotypic differences between the Ningxiang (NX) pigs (an indigenous Chinese breed) and Large White (LW) pigs (a western, lean-type breed). The present work aimed to gain insight into the *longissimus dorsi* muscle transcriptome between the two pig breeds. We investigated the molecular basis of these differences by comparing their transcriptome profiles. RNA-seq technology was used to identify the differentially expressed genes (DEGs) in the *longissimus dorsi* muscle of the NX and LW pigs. We obtained 692 million clean reads using transcriptome sequencing of muscle samples from the two pig breeds. A total of 885 DEGs were identified, including 469 upregulated and 416 downregulated genes in the NX pigs compared with the LW pigs. Using KEGG pathway analysis, it was found that the significant DEGs were mainly enriched in metabolism-related pathways, such as lipid metabolism and biosynthesis, and glucose metabolism or biosynthesis. Quantitative real-time PCR confirmed the differential expression of eight selected DEGs in both pig breeds. qPCR results showed that the RNA-seq results were reliable. Several DEGs were candidate functional genes related to the lipid metabolism, including *CD36*, *LIPE*, *MCAT*, *LPIN1*, *ANGPTL4*, *PPARD*, *SCD*, *INSR*, *MOGAT*, *IGF1*, *AKT2* and *JAK2*. Our results provide a comprehensive basis for the investigation of the differences in transcriptional regulation of the muscles between divergent phenotypes.

Keywords: differentially expressed genes; fatty-type breed; intramuscular fat deposition; lean-type breed

Supported by the National Key R&D Program of China (No. 2021YFD1300403), the National Natural Science Foundation of China (No. U20A2054, No. 32072745), Earmarked Fund for China Agriculture Research System (No. CARS-35), Hunan Provincial Key Research and Development Project (No. 2020NK2031),Tianjin Synthetic Biotechnology Innovation Capacity Improvement Project (No. TSBICIP-CXRC-038).

https://doi.org/10.17221/103/2022-CJAS

Correct animal husbandry for the steady production of quality meat is of particular importance for human health and for the meat processing industry, and helps to ensure sufficient protein production (Ren et al. 2014). In the past decade, high-quality meat has become popular with consumers. Pig breeders have focused on satisfying the demands of the pork production industry primarily through fostering faster pig growth and higher lean meat percentages, resulting in deterioration in meat quality and poorer flavour (Zhao et al. 2009). Intramuscular fat (IMF) is a key meat quality trait correlated with carcass value and nutritional content, and affects energy metabolism and the juiciness, flavour, and tenderness of pork (Xing et al. 2020). Consequently, increasing IMF deposition is a long-term goal in the breeding of lean-type pigs (Shu et al. 2012). Distinct differences have been reported in fat deposition between fat-type and lean-type pigs, including flavour, lean meat percentage, IMF content, and backfat thickness (Xing et al. 2020). Currently, almost nothing is known about the genomic regulation of different IMF deposition traits in fat-type and lean-type pigs, and greater understanding of this issue is important in order to manage the complex biological processes involved.

Ningxiang (NX) pigs (a well-known indigenous Chinese fatty-type breed) have slower growth rates, greater fat deposition characteristics, lower lean meat content, and higher IMF content (7.11% in Ningxiang pig) than conventional LW pigs (IMF content, 2.81%), and are considered to have superior meat quality (Lei et al. 2021). Large White (LW) pigs are a well-known western lean-type breed widely raised worldwide. They are a lean-type pig breed characterized by a fast growth rate and high lean meat content, whose muscle quality is relatively poor (Chen et al. 2017). Modern Large White pigs average < 1% intramuscular fat (IMF), leading to less flavoursome pork meat (Zappaterra et al. 2016). Previous report have indicated that the higher IMF content of fatty NX pigs compared with LW pigs may be due to differences in their colonic gut microbiota (Lei et al. 2021). However, few studies have focused on the differences in the *longissimus dorsi* muscle transcriptome between these breeds. An investigation of the differences between these pig breeds is necessary to understand the functions of differentially expressed genes (DEGs), and their relationships to different meat quality traits.

Comparative transcriptome analysis between breeds is currently widely practiced, and it provides a better understanding of the transcription background of different meat quality traits (Chen et al. 2017). RNA sequencing (RNA-seq) technology has been used in comprehensive analyses of complex traits in animals and it enables a new understanding of gene structures and expression patterns (Chang et al. 2010). RNA-seq not only provides more precise data on transcriptomes than microarrays, but also it can detect lower levels of transcript expression (Gao et al. 2011). RNA-seq provides extremely accurate assessments of transcript levels, and gives highly reproducible results (Xing et al. 2020). In recent years, RNA-seq has been used to analyse transcriptomes in tissues such as skeletal muscle (Yao et al. 2019) and adipose tissue (Ren et al. 2014) in commercial pig breeds. A previous study also used RNA-seq to analyse transcriptome differences in porcine adipose tissue and the *longissimus dorsi* muscle between breeds, in order to reveal the key genes and pathways determining meat quality (Zhao et al. 2009). Overall, RNA-seq is the preferred means of describing and quantifying wholesale changes in the transcriptome, both in human medicine and in farm animal molecular analyses. To date, IMF deposition in NX pigs has only been based on studies of pig feeding regimes and germplasm characteristics (Lei et al. 2021).

Therefore, this study set out to use RNA-seq technology to identify differences in transcriptional expression profiles of the *longissimus dorsi* muscle in NX and LW pigs, and to investigate the molecular basis of these differences. These findings will provide a molecular basis for understanding the fat metabolism in pigs and improve the understanding of the molecular mechanisms of muscle development in these pig breeds.

MATERIAL AND METHODS

Study animals and sample collection

All the experiments involving the use of pigs were carried out according to the Chinese guidelines for animal welfare and experimental protocols, and all experimental protocols were approved by the Committee of Animal Care at Hunan Agricultural University (Changsha, China) (Permit Number: CACAHU 2021-00116). Eight male LW pigs and

https://doi.org/10.17221/103/2022-CJAS

eight male NX pigs were reared on a local breeding farm in Ningxiang city, Hunan Province, China, and they had *ad libitum* access to feed and clean drinking water. LW and NX pigs were fed the maize-soybean basal diets. At 140 days, eight LW pigs and eight NX pigs had an average weight of 99 kg and 49 kg, respectively, they were fasted for 24 h, electrically stunned, and slaughtered. The sample was taken from the *longissimus dorsi* muscle of six animals (three animals per breed which were randomly selected) located at the 3rd/4th last rib. And then, samples were immediately stored at −80 °C until required for transcriptome sequencing and RNA extraction. IMF content in the *longissimus dorsi* muscle was considered to correspond to previously estimated values in NX an LW pigs aged 140 days, i.e. 7.11% and 2.81%, respectively (Lei et al. 2021).

Transcriptome analysis of muscle tissue

Total RNA was extracted from the *longissimus dorsi* muscle samples using a mirVana™ miRNA Isolation Kit (Ambion, Austin, TX, USA), in accordance with the manufacturer's instructions. The integrity of the RNA was checked using an Agilent BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). After the total RNA sample was purified, a cDNA library was prepared using TruSeq Stranded Total RNA with Ribo-Zero Gold Kit (Illumina, San Diego, CA, USA). All of the *longissimus dorsi* muscle sample solutions had RNA quality integrity numbers ≥ 7. RNA libraries were constructed and Solexa sequencing was performed by Shanghai OE Biotech Co., Ltd (Shanghai, China) on an Illumina Genome Analyzer. The libraries were sequenced using the Illumina sequencing platform (HiSeq™ 2500). Raw reads were processed using an NGS QC Toolkit. The reads containing poly-N and the low-quality reads were removed to obtain clean reads. These clean reads were aligned and identified based on the reference genome, Ensembl Sus scrofa 11.1 (http://asia.ensembl.org/Sus_scrofa/Info/Index). The numbers of reads per kilobase of the exon region in gene per million mapped reads were used as the value of the normalized gene expression levels. DEGs were identified using DEGSeq (http://bioconductor.org/packages/stats/bioc/DEGSeq/). $P < 0.05$ and fold change > 2 were set as the thresholds for significantly differential expression.

Gene ontology and Kyoto Encyclopaedia of Genes and Genomes annotation

The DAVID online software was used to analyse the Gene ontology (GO) and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway function annotation of the DEGs. The differentially expressed genes were classified by their molecular function categories, cellular components, and biological processes using GO annotation. GO and KEGG pathways with $P < 0.05$ were considered to be significantly enriched with DEGs.

Integration of protein-protein interaction network

Search Tool for the Retrieval of Interacting Genes (STRING, https://cn.string-db.org/) database is an online tool designed to evaluate protein-protein interaction (PPI) information. STRING v11.5 was used to evaluate the interactive relationships among DEGs, the DEGs were uploaded into STRING database, and the following active prediction methods were employed: neighbourhood, co-expression, gene fusion, experiments, co-occurrence, database, and text mining, with a medium confidence score. Then, PPI networks were visualized by Cytoscape (https://cytoscape.org/) software, and the topological property of the networks was evaluated by it.

Quantitative real-time PCR

Eight genes were chosen to validate the accuracy of the results of the skeletal muscle transcriptome sequencing data, and tested using quantitative real-time polymerase chain reaction (qPCR). qPCR was performed using Luminaris Color HiGreen qPCR Master Mix (Thermo Fisher, Waltham, MA, USA), according to the manufacturer's instructions. All of the PCR reactions were performed in triplicate. The samples used for the real-time PCR assay were the same as those used in the Solexa sequencing experiment. The relative expression level of mRNA was calculated using actin beta as an endogenous reference gene using the $2^{-\Delta\Delta Ct}$ method. The relative gene expression levels of the two pig breeds were compared using a *t*-test with the SAS software v9.0 (SAS Institute, Inc., Cary, NC, USA), and $P < 0.05$

https://doi.org/10.17221/103/2022-CJAS

was considered to indicate a significant difference. The information on the primers is listed in Table 1.

RESULTS

Transcriptome of the longissimus dorsi muscle

A total of six samples (three each from NX and LW pigs) were selected for sequencing. The basic information of sequencing data is also listed in Table 2. 116 302 899 average raw reads were identified in the Large White pigs by transcriptome sequencing (L1, 126 098 692; L2, 125 934 870; L3, 117 461 916 raw clean reads, respectively). 112 549 719 average clean reads were identified in the Large White pigs by transcriptome sequencing (L1, 122 119 016; L2, 122 426 018; L3, 114 087 908 clean reads, respectively). 123 165 159 average raw reads were identified in the Ningxiang pigs by transcriptome sequencing (NX1, 118 277 570; NX2, 102 664 072; NX3, 127 967 054 clean reads, respectively). 119 544 314 average clean reads were identified in the Ningxiang pigs by transcriptome sequencing (NX1, 114 406 306; NX2, 98 919 752; NX3, 124 323 100 clean reads, respectively). In conclusion, the number of clean reads and the comparison results meet the requirements which lay a foundation for the reliability of subsequent data analysis.

Identification and analysis of differentially expressed genes

In order to obtain the putative candidate genes that differed between the two pig breeds, we

Table 1. Primers of genes selected for quantitative PCR confirmation

Gene ID	Gene	Sequences of primers (5'–3')	Product size (bp)
ENSMUSG00000029580	*ACTB*	F: AGAGCAAGAGAGGCATCCTG R: CACGCAGCTCGTTGTAGAAG	111
ENSSSCT00000007507	*AMY2*	F: CCCAGTATGCCCCACAAACCC R: CCTGTACCCCTCCAAATCCTTT	126
ENSSSCT00000025528	*LOC100521789*	F: GGCCCAGTATGCCCCACAAA R: GGGCCCAAATACCGCTCACA	105
ENSSSCT00000008668	*ABAT*	F: AGCGACCACGCACTCCAAAG R: GACAGCGGGCCTCTTCTTGT	134
ENSSSCT00000034991	*IGF1*	F: AGGAGGCTGGAGATGTACTGTG R: CGTTTTCTTGTTGGTAGATGGG	126
ENSSSCT00000003352	*LIPE*	F: GAGATGGAGGATCACTCTGACTC R: CAAGTTGGGTCGGGACTTGT	159
ENSSSCT00000011546	*SCD*	F: TCCCCAAAGCCTGTTCGTCG R: GTGGAAGCCCTCACCCACAG	169
ENSSSCT00000032593	*CD36*	F: TGGGCTGCAATAGAGACTGTGG R: TCCGTGCCTGTTTTAACCCAA	178
ENSSSCT00000032787	*PRKAB2*	F: ATCATGGTGGGGAGCACCGA R: ACCAGCGGATAACAGTGGGC	148

Table 2. Summary of RNA-seq results

Item	L1	L2	L3	Average values (L)	NX1	NX2	NX3	Average values (N)
Raw reads	126 098 692	125 934 870	117 461 916	116 302 899	118 277 570	102 664 072	127 967 054	123 165 159
Clean reads	122 119 016	122 426 018	114 087 908	112 549 719	114 406 306	98 919 752	124 323 100	119 544 314
Q30 (%)	92.22	92.86	92.72	92.60	92.29	92.80	92.72	92.60
GC (%)	53.00	53.00	53.00	51.83	52.00	51.50	52.00	53

L1, L2, L3 = the *longissimus dorsi* muscle samples from three Large White pigs; NX1, NX2, NX3 = the *longissimus dorsi* muscle samples from three Ningxiang pigs

https://doi.org/10.17221/103/2022-CJAS

used DEGSeq software to normalize the number of counts of each sample mRNA. Using the screen criteria of fold change ≥ 2 and $P \leq 0.05$, a total of 885 DEGs were identified, including 469 upregulated and 416 downregulated genes in the NX pigs compared with the LW pigs. All DEGs are listed in Tables S1 and S2 in electronic supplementary material (ESM; for the ESM see the electronic version). The MA plot and the volcano plot of the DEGs are shown in Figures 1A and 1B.

GO and KEGG enrichment analysis of DEGs

The functional classification of all DEGs was ascertained using the GO notation. The GO enrichment analysis in Figure 2 indicated that the DEGs in both pig breeds were mainly involved in biological processes (737 DEGs), the most represented terms were metabolic process (72 DEGs), response to bacterium (six DEGs: *FCGR1A*, *SERPINB9*, *P2RX7*, *CASP1*, *CD14* and *DEFB1*), circadian rhythm (seven DEGs: *ENSSSCT00000003494*, *NFIL3*, *ID4*, *ENSSSCT00000014635*, *TIMELESS*, *FAS* and *PER3*), positive regulation of monocyte chemotaxis (four DEGs: *CCR1*, *CXCL10*, *CCR2* and *CCL2*) and negative regulation of viral genome replication (five DEGs: *OASL*, *RSAD2*, *MX1*, *ISG20* and *ENSSSCT00000029764*). In the cellular component category (72 DEGs), the most represented terms were plasma membrane (17 DEGs), basement membrane (eight DEGs), recycling endosome (eight DEGs) and intracellular membrane-bounded organelle (22 DEGs). In the molecular function category (268 DEGs), the most represented terms were catalytic activity (37 DEGs), double-stranded RNA binding (eight DEGs) and pyridoxal phosphate binding (seven DEGs).

Pathway analysis of the differential mRNAs using the KEGG database was used to discover which differential pathways of different samples may be associated with changes in pathways. The pathways were classified by their correlation of enrichment.

In the top 20 significantly enriched pathways, including carbohydrate digestion and absorption pathway and AMPK signalling pathway and other pathways were highly enriched in NX and LW pigs (Figure 3).

To obtain genes associated with differences in lipid deposition, further analysis of all upregulated and downregulated genes associated with lipid metabolism was performed. The results showed that these DEGs enriched in the four pathways (in-

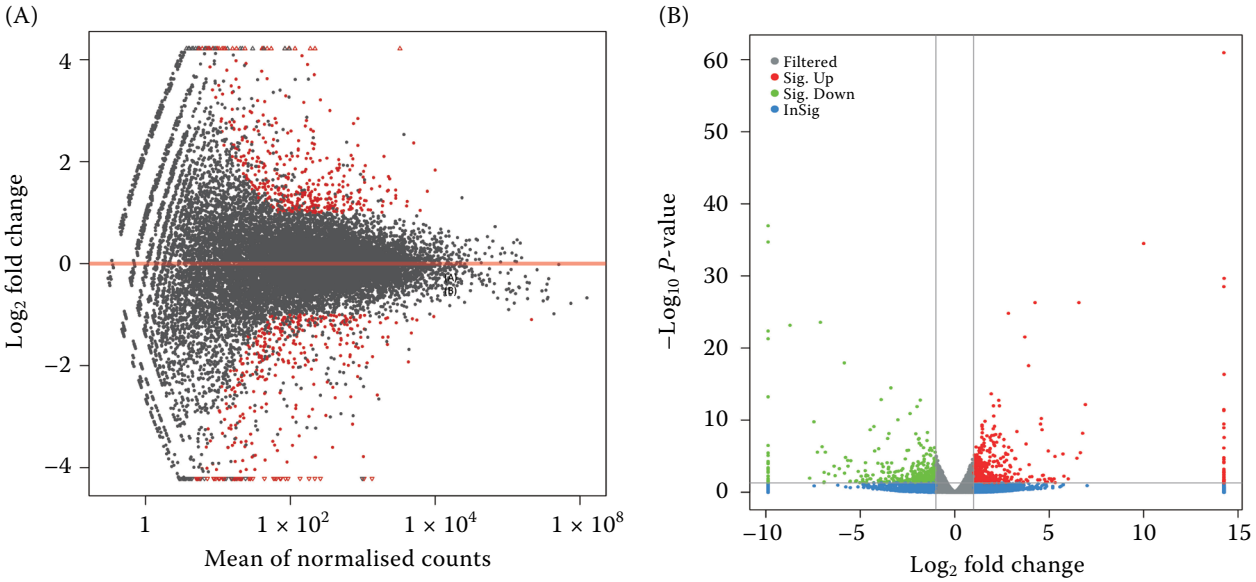

Figure 1. (A) MA plot between Ningxiang and Large White breeds; (B) volcano plot for differentially expressed genes (DEGs)

(A) The *x*-axis is the mean of expression levels of all samples used for comparison after standardization, and the *y*-axis is the $\log_2$ fold change. The differential transcripts highlighted in red are significant (by difference screening criteria). (B) The *x*-axis represents the $\log_2$ (fold change) of all the genes. The *y*-axis represents the $-\log_{10}$ *P*-value. Red represents upregulated DEGs. Blue represents downregulated DEGs of Ningxiang and Large white breeds

https://doi.org/10.17221/103/2022-CJAS

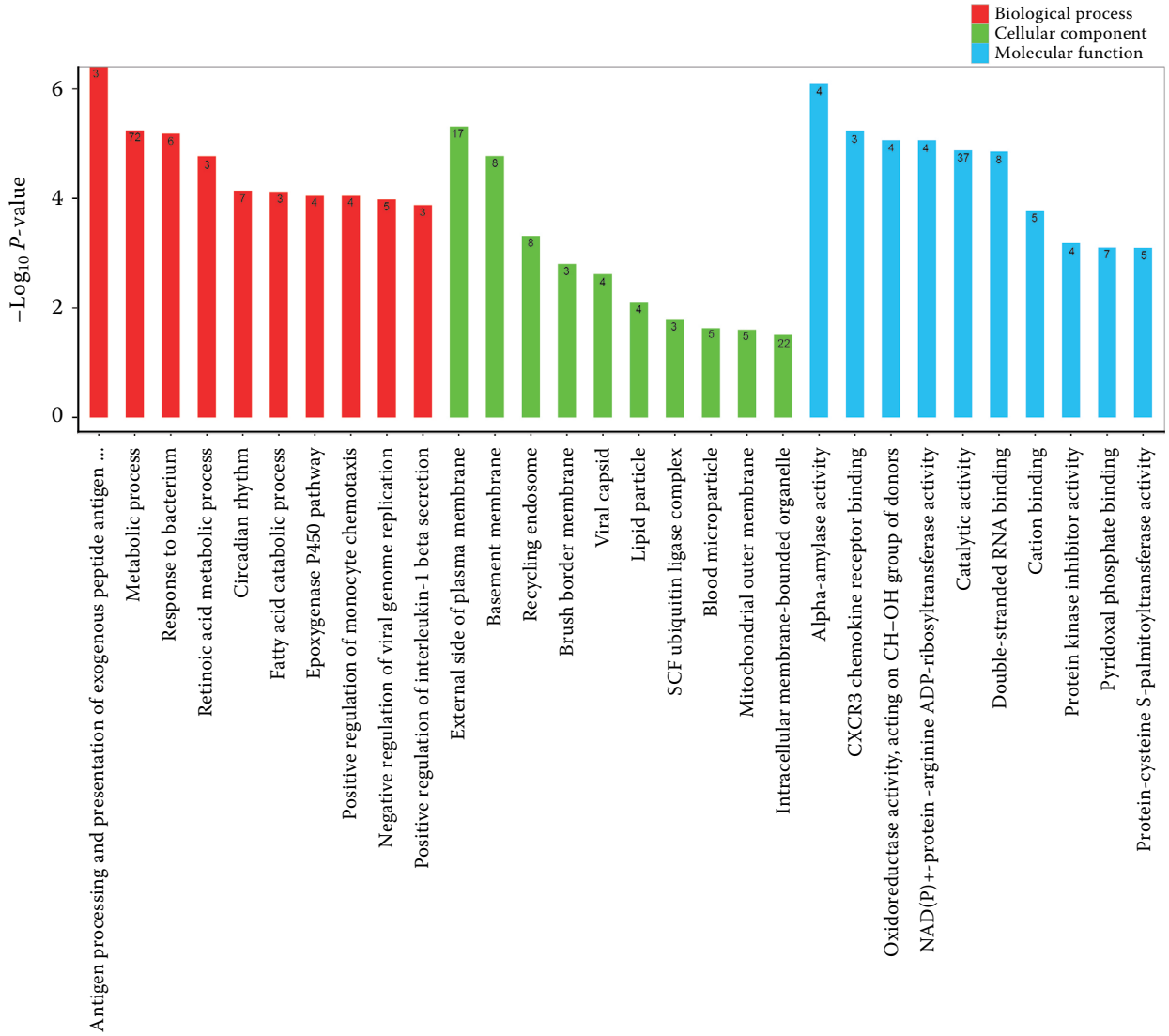

Figure 2. Gene ontology (GO) analysis of differentially expressed genes between the Ningxiang and Large White pigs

cluding fatty acid biosynthesis, glycerolipid metabolism, glycerophospholipid metabolism and PPAR signalling pathway) involved in lipid metabolism were upregulated ($P < 0.05$) and two pathways involved in lipid metabolism had an upregulated trend (adipocytokine signalling pathway, $P = 0.092\ 9$; steroid biosynthesis, $P = 0.064\ 0$) in NX pigs (Table 3). The AMPK signalling pathway was downregulated ($P < 0.05$) in NX pigs (Table 4), and two pathways involved in lipid metabolism had a downregulated trend (steroid biosynthesis, $P = 0.059\ 3$; adipocytokine signalling pathway, $P = 0.059\ 3$). These pathways were mainly associated with the regulation of lipid metabolism and biosynthesis, glucose metabolism and biosynthesis, inflammation or cancer, adipocytokine signalling pathway (*CD36*, and *CAMKK2*), AMPK signalling pathway (*LIPE*, *SCD*, *CD36*, *IGF1*, *CAMKK1* and *CAMKK2*), fatty acid biosynthesis (*MCAT*), glycerophospholipid metabolism (*PCYT1B*, *CRLS1*, *LPIN1*), and peroxisome proliferator-activated receptor (PPAR) signalling pathway (*ANGPTL4*, *CD36* and *PPARD*).

Protein-protein interaction analysis

The screened differentially expressed genes associated with lipid metabolism were analysed by the interaction network; a network of physical and functional protein-protein interactions (PPI) was built using STRING v11.5 online software. In the core of the protein-protein interaction net-

https://doi.org/10.17221/103/2022-CJAS

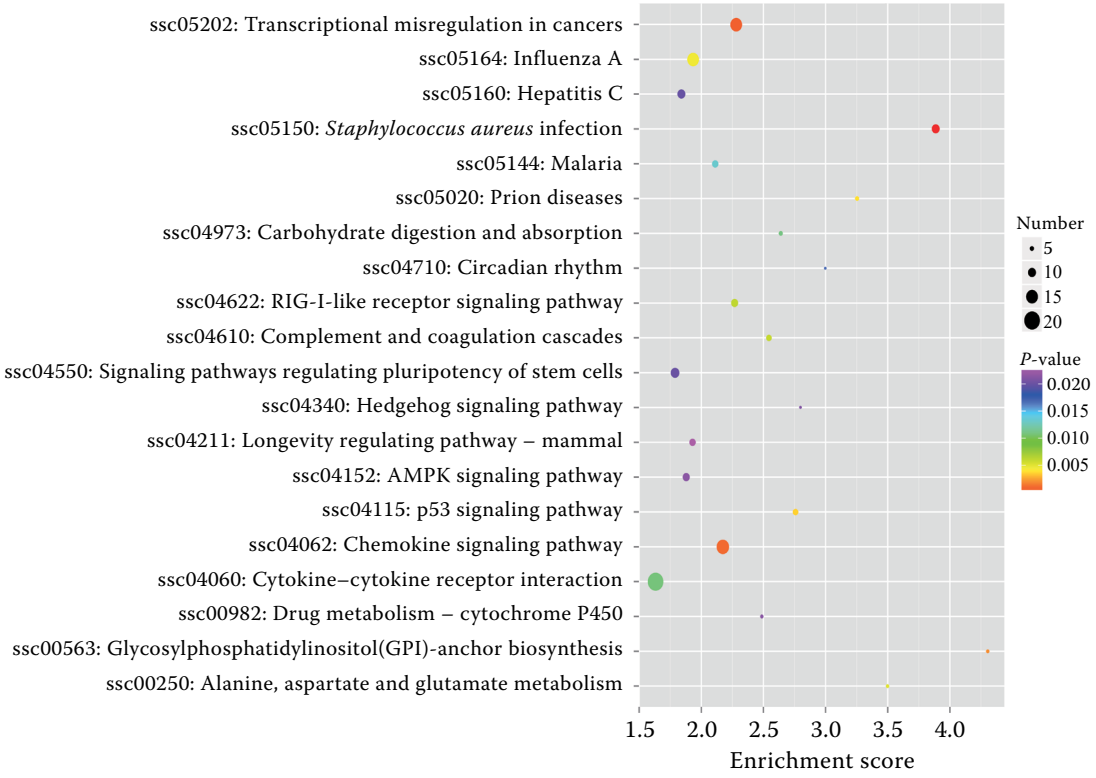

Figure 3. Enriched KEGG pathways of differentially expressed genes

work, genes belonged to more than one module (Figure 4). Dense network associations were also observed around lipase E (*LIPE*), stearoyl-CoA desaturase (*SCD*), lipin 1 (*LPIN1*), pro-opiomelanocortin (*POMC*), insulin-like growth factor 1 (*IGF1*) and insulin receptor (*INSR*).

Table 3. List of DEGs involved in pathways related with fat metabolism (upregulation in Ningxiang pigs)

Pathway ID	Pathway	Up-regulated genes	*P*-value
ssc04920	adipocytokine signaling pathway	ENSSSCT00000032593 (*CD36*) ENSSSCT00000010758 (*CAMKK2*) ENSSSCT00000032500 (*POMC*)	0.092 9
ssc04152	AMPK signaling pathway	ENSSSCT00000003352 (*LIPE*) ENSSSCT00000032593 (*CD36*) ENSSSCT00000010758 (*CAMKK2*) ENSSSCT00000034530 (*INSR*)	0.064 0
ssc00061	fatty acid biosynthesis	ENSSSCT00000000033 (*MCAT*)	0.017 8
ssc00561	glycerolipid metabolism	ENSSSCT00000017667 (*MOGAT1*) ENSSSCT00000030171 (*LPIN1*) ENSSSCT00000009435 (*LPIN1*)	0.017 2
ssc00564	glycerophospholipid metabolism	ENSSSCT00000035302 (*PCYT1B*) ENSSSCT00000007717 (*CRLS1*) ENSSSCT00000030171 (*LPIN1*) ENSSSCT00000009435 (*LPIN1*)	0.009 5
ssc03320	PPAR signaling pathway	ENSSSCT00000014853 (*ANGPTL4*) ENSSSCT00000032593 (*CD36*) ENSSSCT00000001714 (*PPARD*)	0.036 3

https://doi.org/10.17221/103/2022-CJAS

Table 4. List of DEGs involved in pathways related with fat metabolism (downregulation in Ningxiang pigs)

Pathway ID	Pathway	Down-regulated genes	*P*-value
ssc04152	AMPK signaling pathway	ENSSSCT00000011546 (*SCD*) ENSSSCT00000035532 (*IGF1*) ENSSSCT00000034991 (*IGF1*) ENSSSCT00000019449 (*CAMKK1*) ENSSSCT00000033477 (*AKT2*)	0.048 3
ssc04920	adipocytokine signaling pathway	ENSSSCT00000034494 (*JAK2*) ENSSSCT00000019449 (*CAMKK1*) ENSSSCT00000034165 (*IKBKG*) ENSSSCT00000033477 (*AKT2*)	0.059 3
ssc00100	steroid biosynthesis	ENSSSCT00000027336 (*DHCR24*)	0.063 3
ssc04923	regulation of lipolysis in adipocyte	ENSSSCT00000031113 (*TSHR*) ENSSSCT00000033477 (*AKT2*)	0.229 0
ssc01212	fatty acid metabolism	ENSSSCT00000011546 (*SCD*)	0.265 6
ssc00561	glycerolipid metabolism	ENSSSCT00000014305 (*TKFC*)	0.345 7
ssc04310	Wnt signaling pathway	ENSSSCT00000025742 (*WNT11*) ENSSSCT00000001551 (*CSNK2B*)	0.395 4
ssc03320	PPAR signaling pathway	ENSSSCT00000011546 (*SCD*)	0.456 5

Validation of differentially expressed genes

Eight differentially expressed genes were chosen at random to validate the accuracy of the RNA-seq using qPCR. The results showed that the fold changes of the eight genes in the qPCR and in the RNA-seq showed the same trends (Figure 5). Thus, the qPCR results showed that the RNA-seq results were reliable.

DISCUSSION

The objective of this study was to evaluate and compare the inherited elements of meat quality differences and transcriptome characteristics of the *longissimus dorsi* muscle between two pig phenotypes. A total of 885 DEGs were discovered in the two pig breeds, including 469 upregulated and

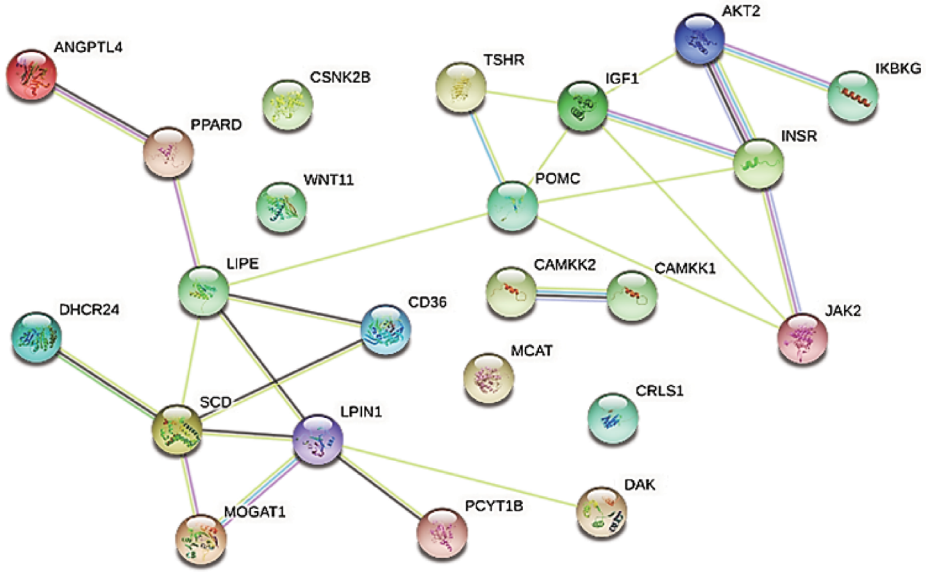

Figure 4. Protein-protein interaction network of differentially expressed genes analysed by STRING
Each coloured line represents different evidence for each interaction: red, fusion; green, neighbourhood; blue, co-occurrence; purple, experimental; yellow, text mining; light blue, database; black, co-expression

https://doi.org/10.17221/103/2022-CJAS

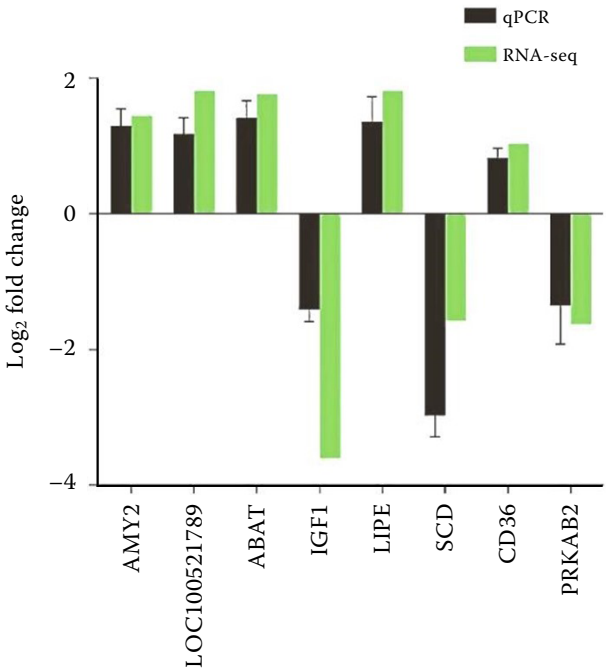

Figure 5. Eight DEGs were validated by qPCR (black) and RNA-seq (green) in the *longissimus dorsi* muscle tissue

416 downregulated genes, revealing the different biological processes and differentially expressed genes regulating the lipid metabolism. A previous study identified numerous DEGs in the *longissimus dorsi* muscle of lean-type and fatty-type breeds using transcriptome sequencing, and some of these DEGs (such as *SCD* and *ANGPTL4*) overlap with the results of this study and the differences we found between the NX and LW pig breeds (Chen et al. 2017). Furthermore, several DEGs concerned lipid metabolic processes, which may account for the completely different ones in weights and body composition (especially higher IMF content) in NX pigs compared with LW pigs.

One previous study showed that the calcium/calmodulin-dependent protein kinase 2 gene (*CAMKK2*) can be considered a key gene for the regulation of critical metabolic processes in adiposity and glucose homeostasis (Musi and Goodyear 2002). Obesity and glucose intolerance in mice fed a high-fat diet were modulated by the low expression or loss of the *Camkk2* gene, suggesting that *Camkk2* regulates the lipid metabolism (Ramos-Lopez et al. 2018). In pigs, the *CAMKK2* gene has been reported to affect pathways related to the AMP-activated protein kinase (AMPK) signalling pathway in meat quality traits, and that the *CAMKK2* gene was downregulated in Min pigs whose IMF content is higher than in LW pigs (Foll et al. 2015). In this study, the expression level of *CAMKK2* had an upregulated trend in NX pigs, indicating that the *CAMKK2* gene may not be a candidate gene for the mediation of lipid regulation in NX pigs. Therefore, the differences in the underlying molecular mechanisms need further exploration in the NX and LW breeds.

The cluster of differentiation 36 (CD36), also called fatty acid translocase, functions as a class B scavenger receptor that, when specifically combined with oxidized low-density lipoprotein, regulates the levels of free fatty acids and triglycerides in plasma (Thorne et al. 2006). According to Sfeir et al. (1997), the level of CD36 affects the transport of membrane long-chain fatty acids and subsequently their binding and transport. Foll et al. (2015) discovered that the *CD36* gene regulates energy and glucose homeostasis and fat deposition in the adipose tissue of rats, and that its overexpression leads to higher levels of subcutaneous fat. INSR transduces the cell surface signal via phosphoinositide-3-kinase-AKT pathways, or translocates to the nucleus and binds to the promoters to regulate genes associated with insulin actions including *de novo* lipogenesis (Saltiel and Kahn 2001). Monoacylglycerol O-acyltransferase (*MOGAT*) expressed extensively in various tissues, and had relatively higher expression levels in the lipid metabolism organs (Foll et al. 2015). In this study, we found that the *CD36*, *INSR*, and *MOGAT* genes were highly expressed in the *longissimus dorsi* muscle of NX pigs and this suggests that *CD36*, *INSR*, and *MOGAT* genes are the candidate genes for the regulation of fat deposition.

This report showed that the expression of the *LIPE* gene was positively correlated with the intramuscular fat content of pigs (Zappaterra et al. 2016). *LIPE* is expressed in adipose tissue, and catalyzes the intracellular triacylglycerol stored there to free fatty acids (Xue et al. 2015). The *LIPE* gene is involved in antilipolysis and participates in lipid homeostasis. It has been reported that the expression of the *LIPE* gene and protein differs between Chinese Wujin pigs (with a higher IMF content) and Western Landrace pigs (with a lower IMF content), indicating that it may be a candidate gene affecting intramuscular lipid deposition (Zhao et al. 2009). The *LIPE* gene is expressed in adipose tissue, and cleaves free fatty acids from the intracellular triacylglycerol stored there (Shu et al. 2012). Because the *CD36* and *LIPE*

https://doi.org/10.17221/103/2022-CJAS

genes participate in the AMPK signalling pathway, their differential expression may correspond to the differences in meat quality traits between NX and LW pigs. Therefore, the upregulated trend and their levels may promote lipid deposition in the *longissimus dorsi* muscle of NX pigs.

LPIN1 gene plays a critical role in adipocyte differentiation and lipid metabolism (Assaily et al. 2011). *LPIN1* gene expression in adipose tissue has been associated with the regulation of adiposity and metabolic profiles in the Chinese human population (Chang et al. 2010). Additionally, *LPIN1* affects phospholipid phosphorylase activity, which is the rate-limiting step in animal fat formation. It catalyzes phospholipid acid to form diglycerol, which plays a key role in the formation of triglyceride, and is closely related to the formation of animal fat (Miranda et al. 2010). It is also required for normal adipocyte differentiation and induction of adipogenic gene transcription, so it is a candidate gene for the deposition of intramuscular fat in pigs (He et al. 2009). In mice, overexpression of *Lpin1* induced higher levels of adiposity (Phan and Reue 2005). In this study, *LPIN1* gene expression levels were upregulated, indicating that high expression levels of the *LPIN1* gene probably have positive effects on lipid deposition in the *longissimus dorsi* muscle of pigs.

Angiopoietin-like protein 4 (*ANGPTL4*) is a target gene for the peroxisome proliferator activated receptor gamma (*PPARG*), which is involved in adipogenesis and promotes adipocyte differentiation and regulates the expression of specific adipocyte genes (Phan and Reue 2005). *ANGPTL4* is an important regulator of triacylglycerol metabolism and an inhibitor of lipoprotein lipase, and higher expression of *Angptl4* can regulate the fat metabolism in high-fat induced mice by inhibiting lipoprotein lipase activity (Gao et al. 2019). Ren et al. (2014) revealed that the expression of *ANGPTL4* genotypes showed a difference in IMF content between Meishan and Large White pigs, suggesting that the *ANGPTL4* genotypes affected the moisture content of the *longissimus dorsi*, meat marbling of the *longissimus dorsi*, IMF of the *longissimus dorsi*, and pH of the *longissimus dorsi*. A higher expression of *ANGPTL4* in fatty pigs increases effect on fat deposition, compared with lean pigs (Feng et al. 2006). These results suggest that the *ANGPTL4* gene might play a vital role during lipid deposition in pigs.

Nuclear receptor PPAR is a key regulator of *CD36*, and plays an important role in scavenging cell lipids by regulating the expression of genes related to lipid oxidation and cell proliferation (Barish et al. 2006). The peroxisome proliferator-activated receptor delta (PPARD) is distributed in fatty acid metabolism-related tissues including the liver, adrenal glands and adipose, and can affect fat deposition energy uncoupling, mitochondrial activity, and respiration (Mehla et al. 2014). PPARD is a promising candidate protein for pig carcass traits (Xu et al. 2018). Shu et al. (2012) also reported that the PPARD was upregulated in pigs with higher fat deposition traits. Malonyl-CoA-acyl carrier protein transacylase (MCAT) is the rate-limiting enzyme required for long-chain fatty acid biosynthesis and it has been shown to be positively correlated with fatty acid synthesis (Arthur et al. 2009). Thus, *CD36* and *MCAT* genes display an increased expression in NX pigs compared with LW pigs, suggesting that the ability to respond to fat deposition increases in NX pigs.

In the lipid metabolism pathway, we saw lower expression of *SCD* and higher expression of *MCAT* in NX pigs. The *SCD* gene is an important component of the leptin metabolic pathway, and leptin inhibits the obese phenotype by downregulating *SCD* expression (Miyazaki et al. 2003). Similarly, the expression level of the *SCD* gene in Northeast pigs was significantly lower than in LW pigs, indicating that the absolute content of *SCD* in LW pigs is still very high (Gao et al. 2011). IGF1 was reported that it could decrease the lipid metabolism level in recreational athletes, suggesting that IGF1 is involved in adipocyte regulation, supporting our finding (Guha et al. 2011). Furthermore, threonine kinase 2 (Akt2) is required for hepatic lipid accumulation in obese, insulin-resistant states induced by either leptin-deficiency or high-fat diet feeding (Leavens et al. 2009). Chang et al. (2010) indicated that Janus kinase 2 (JAK2) exerted main regulating actions of leptin on the lipid metabolism. In the present study, the expression levels of the *SCD*, *IGF1*, *AKT2* and *JAK2* genes show differences between NX and LW pigs, suggesting that those genes may be involved in the fat metabolism in pigs.

CONCLUSION

In this study, transcriptome sequencing was performed to identify the biological processes underlying the differences in the *longissimus dorsi* muscle

https://doi.org/10.17221/103/2022-CJAS

between NX and LW pigs. Using KEGG pathway analysis, we found that the enriched DEGs were mainly those regulating metabolism-related pathways, such as lipid metabolism and biosynthesis, glucose metabolism, or biosynthesis. These results provide a comprehensive overview for the investigation of the differences in transcriptional regulation in muscles between divergent phenotypes, and several DEGs can be considered as functional candidate genes related to the lipid metabolism, including *CD36*, *LIPE*, *MCAT*, *LPIN1*, *ANGPTL4*, *PPARD*, *SCD*, *INSR*, *MOGAT*, *IGF1*, *AKT2* and *JAK2*. However, the function of these genes remains unclear. Further studies are required to investigate the roles of these candidate genes in order to develop genetic breeding programs and improve the production performance of pigs and other livestock.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Arthur CJ, Williams C, Pottage K, Ploskon E, Findlow SC, Burston SG, Simpson TJ, Crump MP, Crosby J. Structure and malonyl CoA-ACP transacylase binding of streptomyces coelicolor fatty acid synthase acyl carrier protein. ACS Chem Biol. 2009 Aug 21;4(8):625-36.

Assaily W, Rubinger DA, Wheaton K, Lin Y, Ma W, Xuan W, Brown-Endres L, Tsuchihara K, Mak TW, Benchimol S. ROS-mediated p53 induction of Lpin1 regulates fatty acid oxidation in response to nutritional stress. Mol Cell. 2011 Nov 4;44(3):491-501.

Barish GD, Narkar VA, Evans RM. PPARδ: A dagger in the heart of the metabolic syndrome. J Clin Invest. 2006 Mar;116(3):590-7.

Chang YC, Chang LY, Chang TJ, Jiang YD, Lee KC, Kuo SS, Lee WJ, Chuang LM. The associations of LPIN1 gene expression in adipose tissue with metabolic phenotypes in the Chinese population. Obesity. 2010 Jan;18(1):7-12.

Chen W, Fang GF, Wang SD, Wang H, Zeng YQ. Longissimus lumborum muscle transcriptome analysis of Laiwu and Yorkshire pigs differing in intramuscular fat content. Genes Genom. 2017 Jul 10;39(7):759-66.

Feng SQ, Chen XD, Xia T, Gan L, Qiu H, Dai MH, Zhou L, Peng Y, Yang ZQ. Cloning, chromosome mapping and expression characteristics of porcine ANGPTL3 and -4. Cytogenet Genome Res. 2006;114(1):44-9.

Foll CL, Dunn-Meynell AA, Levin BE. Role of FAT/CD36 in fatty acid sensing, energy, and glucose homeostasis regulation in DIO and DR rats. Am J Physiol Regul Integr Comp Physiol. 2015 Feb 1;308(3):R188-98.

Gao Y, Zhang YH, Jiang H, Xiao SQ, Wang S, Ma Q, Sun GJ, Li FJ, Deng Q, Dai LS, Zhao ZH, Cui XS, Zhang SM, Liu DF, Zhang JB. Detection of differentially expressed genes in the longissimus dorsi of northeastern indigenous and large white pigs. Genet Mol Res. 2011 May 3;10(2):779-91.

Gao J, Ding G, Li Q, Gong L, Huang J, Sang Y. Tibet kefir milk decreases fat deposition by regulating the gut microbiota and gene expression of Lpl and Angptl4 in high fat diet-fed rats. Food Res Int. 2019 Jul 1;121:278-87.

Guha N, Erotokritou-Mulligan I, Nevitt S, Francis M, Bassett E, Sonksen P, Holt R. The effects of recombinant human IGF1/IGF binding protein-3 on lipid and glucose metabolism in recreational athletes. Clin Endocrinol. 2011 Apr;94(4):551-62.

He XP, Xu XW, Zhao SH, Fan B, Yu M, Zhu MJ, Li CC, Peng ZZ, Liu B. Investigation of Lpin1 as a candidate gene for fat deposition in pigs. Mol Biol Rep. 2009 May 1; 36(5):1175-80.

Leavens KF, Easton RM, Shulman GI, Previs SF, Birnbaum MJ. Akt2 is required for hepatic lipid accumulation in models of insulin resistance. Cell Metab. 2009 Nov 4; 10(5):405-18.

Lei L, Wang Z, Li J, Yang H, Yin Y, Tan B, Chen J. Comparative microbial profiles of colonic digesta between Ningxiang pig and Large White pig. Animals. 2021 Jun 23; 11(7):1862-73.

Mehla K, Magotra A, Choudhary J, Singh AK, Khan F. Genome-wide analysis of the heat stress response in Zebu (Sahiwal) cattle. Gene. 2014 Jan 10;533(2):500-7.

Miranda M, Escote X, Ceperuelo-Mallafre V, Megia A, Caubet E, Naf S, Gomez JM, Gonzalez-Clemente JM, Vicente V, Vendrell J. Relation between human LPIN1, hypoxia and endoplasmic reticulum stress genes in subcutaneous and visceral adipose tissue. Int J Obes. 2010 Apr;34(4):679-86.

Miyazaki M, Jacobson MJ, Man WC, Cohen P, Asilmaz E, Friedman JM, Ntambi JM. Identification and characterization of murine SCD4, a novel heart-specific stearoyl-coa desaturase isoform regulated by leptin and dietary factors. J Biol Chem. 2003 Sep 5;278(36):33904-11.

Musi N, Goodyear LJ. Targeting the AMP-activated protein kinase for the treatment of type 2 diabetes. Curr Drug Targets Immune Endocr Metabol Disord. 2002 Jul; 2(2):119-27.

Phan J, Reue K. Lipin, a lipodystrophy and obesity gene. Cell Metab. 2005 Jan;1(1):73-83.

Ramos-Lopez O, Samblas M, Milagro FI, Zulet MA, Martinez JA. Association of low dietary folate intake with

https://doi.org/10.17221/103/2022-CJAS

lower CAMKK2 gene methylation, adiposity, and insulin resistance in obese subjects. Nutr Res. 2018 Feb;50:53-62.

Ren ZQ, Wu WJ, Liu WH, Zheng R, Xiong YZ. Differential expression and effect of the porcine ANGPTL4 gene on intramuscular fat. Genet Mol Res. 2014 Apr 16;13(2):2949-58.

Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature. 2001 Dec 13; 414(6865):799-806.

Sfeir Z, Ibrahimi A, Amri E, Grimaldi P, Abumrad N. Regulation of FAT/CD36 gene expression: Further evidence in support of a role of the protein in fatty acid binding/transport. Prostag Leukotr Ess. 1997 Jul;57(1):17-21.

Shu PW, Laurin N, Himms-Hagen J, Rudnicki MA, Mitchell GA. The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice. Obes Res. 2012 Feb; 9(2):119-28.

Thorne RF, Zhang X, Song C, Jin B, Burns GF. Novel immunoblotting monoclonal antibodies against human and rat CD36/fat used to identify an isoform of CD36 in rat muscle. DNA Cell Biol. 2006 May;25(5):302-11.

Xing Y, Wu X, Xie C, Xiao D, Zhang B. Meat quality and fatty acid profiles of Chinese Ningxiang pigs following supplementation with N-carbamylglutamate. Animals. 2020 Jan 6;10(1): 11 p.

Xu J, Wang C, Jin E, Gu Y, Li S, Li Q. Identification of differentially expressed genes in longissimus dorsi muscle between Wei and Yorkshire pigs using RNA sequencing. Genes Genom. 2018 Apr;40(4):413-21.

Xue W, Wang W, Jin B, Zhang X, Xu X. Association of the ADRB3, FABP3, LIPE, and LPL gene polymorphisms with pig intramuscular fat content and fatty acid composition. Czech J Anim Sci. 2015 Apr;60(2):60-6.

Yao C, Pang D, Lu C, Xu A, Huang P, Ouyang H, Yu H. Data mining and validation of AMPK pathway as a novel candidate role affecting intramuscular fat content in pigs. Animals. 2019 Apr 1;9(4): 16 p.

Zappaterra M, Deserti M, Mazza R, Braglia S, Zambonelli P, Davoli R. A gene and protein expression study on four porcine genes related to intramuscular fat deposition. Meat Sci. 2016 Nov 1;121:27-32.

Zhao SM, Ren LJ, Chen L, Zhang X, Cheng ML, Li WZ, Zhang YY, Gao SZ. Differential expression of lipid metabolism related genes in porcine muscle tissue leading to different intramuscular fat deposition. Lipids. 2009 Nov 1;44(11):1029-37.

Received: June 17, 2022
Accepted: November 10, 2022
Published online: November 29, 2022