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RNA-Seq Reveals Differentially Expressed Genes and Pathways Affecting Intramuscular Fat Metabolism in Huangshan Black Chicken Population

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Abstract

Intramuscular fat (IMF) plays an important role in meat quality due to its positive correlation with juiciness, tenderness, and flavor. However, for chickens, the molecular mechanisms underlying IMF deposition in thigh muscle have not yet been determined. Here, to identify candidate genes and signaling pathways related to IMF deposition, we deeply explored the chicken transcriptome from thigh muscles of Huangshan Black Chickens with extremely high and low phenotypic values for intramuscular fat content. A total of 128 genes differentially expressed genes (DEGs) were detected, of which 94 were up-regulated and 34 were down-regulated. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways revealed these DEGs (including *FABP4*, *G0S2*, *PLIN1*, *SCD1*, *LFABP*, *SLC1A6*, *SLC45A3*, *ACSBG1*, *LY86*, *ST8SIA5*, *SNAI2*, *HPGD*, *EDN2*, and *THRSP*) were significantly enriched in lipid biosynthetic process, steroid biosynthetic and metabolic process, fatty acid metabolic process, and regulation of unsaturated fatty acid metabolic pathways. Additionally, we concluded an interaction network related to lipid metabolism, which might be contributed to the IMF deposition in chicken. Overall, we proposed some new candidate genes and interaction networks that can be associated with IMF deposition and used as biomarkers in meat quality improvement.

Keywords: meat quality, IMF deposition, thigh muscle, chicken transcriptome, interaction networks

1. Introduction

During the past decades, the breeding of meat type poultry has been predominantly focused on increasing growth rate and yields of breast and thigh meat. Although the impressive progress made in these meat quality traits, there were some poor performances, such as larger fiber diameters, lower intramuscular fat, and higher proportion glycolytic fibers, which seriously decreased sensory acceptability for consumers (Du et al., 2010). It is an ongoing challenge to maintain growth rate meanwhile improve meat quality. As a main determinant of meat quality, the deposition of intramuscular fat (IMF) plays an important role in flavor of meat and can dramatically promote tenderness of meat.

Previous studies have discovered some important quantitative trait loci (QTL) associated with chicken IMF, which are mainly located on chromosomes 1, 2, 5, 23 (Zhao et al., 2007; Sarsenbek et al., 2013; Zhang et al., 2015). Additionally, a number of genes including *CD36*, *ACC*, and *DGAT2* (Jeong et al., 2012), *FABP* (Serao et al., 2011), *DGAT1* (Li et al., 2013), *LPL* (Zhang et al., 2015), and *SLC27A1* (Qiu et al., 2017) were also recognized as candidate genes for IMF in chickens. However, the metabolic pathways underlying IMF deposition is very complicated, the molecular mechanisms affecting IMF remains poorly understood.

With the development of high-throughput sequencing technologies, especially RNA-Seq has been widely utilized to explore potential candidate genes which affect important economic traits in animals. Although, previous studies have analyzed the transcriptome of chicken breast muscle (Cui et al., 2012), skeletal muscle (Ye

et al., 2014) and thigh muscle (Cui et al., 2018) utilizing microarrays, and identified some potential candidate genes that influence IMF deposition, no further transcriptome studies in chickens have been taken in identical breed with distinct IMF levels.

As an indigenous breed in China, the Huangshan Black Chicken has a distinct appearance and quality in meat products. The difference in IMF content of thigh muscles makes them as great materials to understand the molecular mechanism of IMF deposition in chickens. In the present study, we used RNA-Seq technology to examine differentially expressed genes (DEGs) in thigh muscle tissues between two groups of Huangshan Black Chickens with extremely high and low phenotypic values of IMF content. We then proposed some new candidate genes and a gene network that can be related to IMF deposition by conducting integrated analysis. Thus, the elucidation of the precise molecular mechanisms underlying IMF traits in Huangshan Black chickens will have both economic and biological consequences.

2. Method and Methods

2.1 Ethics Statement

All animal procedures were authorized by the Institutional Animal Care and Use Committee (IACUC) of Hefei University of Technology (Permit Number: DK838). In the present study, animals were sacrificed as necessary to ameliorate suffering.

2.2 Animals and Sample Collection

Huangshan Black Chickens (Anhui conservation farm for Huangshan Black Chicken, Huangshan, China) with the same genetic background were maintained in free-ranging flocks in a standardized farm, using a diet as: maize 64.0%, wheat bran 16.0%, full-fat soybean 10.0%, fish meal 5.0%, feed yeast powder 2.0%, bone meal 1.5%, inorganic additives 0.7%, Lysine 0.3%, salt 0.3%, Methionine 0.2%. Ten male chickens with an average weight of 1.82 kg at 120 days old were selected randomly according to our detection. To keep the environment factors identical, feed and water were provided ad libitum during the experiment.

All the chickens were fasted for 12 h, and weighed before being killed by stunning and exsanguination. The thigh muscle samples from the left leg of the chickens were collected within 30 min after slaughter. The samples for each chicken were were snap-frozen and stored at -80 °C before analysis. Meanwhile, sufficient samples were minced and kept at -20 °C for IMF analysis.

2.3 IMF Measurement

IMF content of thigh muscle was determined by Soxhlet extraction according to previous studies (Folch and Lees, 1957) and expressed as percentages of the muscle, on the basis of the dry weight.

2.4 RNA Isolation and Validation

Total RNA of the thigh muscle samples was extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After the quality verification on gel electrophoresis, the concentration and purity of the RNA samples were assessed on a NanoPhotometer® spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The integrity of RNA was assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, USA).

2.5 RNA Sequencing

With a final 2.0 μg/μl concentration, RNA from each sample was pooled based on the IMF content. A total of 3μg RNA from per pooled sample was used as the input material for RNA sample preparations. Based on the manufacturer's instructions, the transcriptome library was constructed using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA). Furthermore, TruSeq PE Cluster Kit v3-cBot-HS (Illumina) was used to cluster the index-coded samples on a cBot Cluster Generation System. After cluster generation, the library preparations were sequenced using an Illumina HiSeq 2000 platform, which was followed by FASTQ file generation and the failed reads elimination by CASAVA ver.1.8.2 (Illumina).

2.6 Sequencing Data Analysis

Using CASAVA ver.1.8.2 (Illumina), the sequencing-derived raw images were transformed into raw reads by base calling. After obtained the raw reads, we removed reads containing low quality reads, adapter and reads containing ploy-N to get clean data through in-house perl scripts. Additionally, the description statistics for the clean data, such as Q20, Q30, and GC-content were calculated for high-quality downstream analysis. The clean data with high quality were used for the downstream analyses.

2.7 Reads Mapping

Based on the reference genome, only these reads with a perfect match or one mismatch were further analyzed and annotated. The clean reads were mapped to the reference genome of chicken (version UMD 4.1) using Tophat2 software (version 2.1.0). The index of the reference genome was built using Bowtie v2.2.3 and paired-end clean reads for each individual chicken were aligned to the reference genome using TopHat v2.0.12. In addition, HTSeq v0.6.1 was used to count the reads numbers mapped to each gene.

2.8 Differential Expression Analysis

Differential expression analysis of different groups (the high and low groups with phenotypic values for IMF content) was identified using the DESeq R package (1.10.1) based on the negative binomial distribution. Furthermore, the Hochberg and Benjamini method was used to adjust the p-values for controlling the false discovery rate (Benjamini and Hochberg, 1995). Genes with a FDR value < 0.05 and jlog2-fold changej > 2 were assigned as differentially expressed.

2.9 Functional Enrichment Analysis

GO and KEGG pathway enrichment analyses of the DEGs were implemented by the Database for Annotation, Visualization and Integrated Discovery (DAVID) website (Huang et al., 2007). GO terms and KEGG pathways with a hypergeometric test from the R package ($P < 0.1$, FDR-adjusted) were considered significantly enriched among the DEGs. Pathways with fewer than three known chicken genes were discarded.

To validate the repeatability and reproducibility of the sequencing results, qRT-PCR was carried out to detect 10 randomly selected DEGs. Primers were designed via Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/input.htm) and are shown in Supplementary Table S1. qRT-PCR was carried out in triplicate with the LightCycler® 480 SYBR Green I Master Kit (Roche Applied Science, Penzberg, Germany) in a 15 μ L reaction on a ABI7500 (Applied Biosystems Inc., USA), using the following program: 95 °C for 10 min; 40 cycles of 95 °C for 10 s, 60 °C for 34 s, and 72 °C for 10 s; 72 °C for 6 min. The mRNA levels of the DEGs were normalized by the housekeeping genes GAPDH and β-actin, and the relative gene expression values were calculated using the $2^{-\Delta\Delta Ct}$ method. Finally, the correlations between RNA-Seq for 10 genes and the mRNA expression level from qRT-PCR were estimated using R (V3.2).

3. Results

3.1 Differences in IMF Content

The IMF content of 20 samples in thigh tissues was detected using soxhlet extraction method and the data were shown in Table 1, respectively. Of these, according to the value of the IMF content, the pooled RNA of sample 1-3 and sample 4-6 were selected as IMFL1 and IMFL2 while sample 7-9 and sample 10-12 as IMFH1 and IMFH2 to explore the chicken transcriptome by paired-end RNA sequencing.

Table 1. Analysis of IMFs in thigh muscle of Huangshan Black chickens

Sample	IMF content (%)	Group	Mean±SD (%)
sample1	2.72		
sample 2	2.63	IMFL1	2.69±0.05
sample 3	2.72		
sample 4	2.28		
sample 5	2.18	IMFL2	2.22±0.05
sample 6	2.20		
sample 7	3.80		
sample 8	3.88	IMFH1	3.86±0.06
sample 9	3.90		
sample 10	3.90		
sample 11	3.94	IMFH2	3.93±0.03
sample 12	3.95		
sample 13	2.93		
sample 14	3.43		
sample 15	3.71		
sample 16	3.54		
sample 17	2.86		
sample 18	3.15		
sample 19	2.99		
sample 20	3.22		

Note. IMFH1and IMFH2 means samples with extremely high phenotypic values for intramuscular fat content; IMFL1and IMFL2 means extremely low phenotypic values, respectively.

3.2 RNA Sequencing of Thigh Muscle Tissue

We acquired a total of 240.02 million clean reads with an average of 60.01 million (range, 57.85 to 62.40 million) for each sample (Table 2). Alignment of the sequence reads against the chicken reference genome UMD 4.1 yielded 71.39-72.61% of uniquely aligned reads across the four samples, of which 76.1-76.8% fell in annotated exons, 5.9-7.7% were located in introns, and 16.1-17.2% fell in intergenic regions (Supplementary Figure S1). The data sets analyzed are available in the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank) and the BioProject ID is PRJNA471361. Furthermore, the correlation coefficient (R^2) between the four individuals within the IMFH and IMFL groups was calculated based on the FRPM value of each sample and was shown to be 0.937 and 0.964, respectively, indicating that the similarity of the two biological samples within each group was sufficiently high (Supplementary Figure S2).

Table 2. Basic sequencing data for each sample

Sample_name	IMFH1	IMFH2	IMFL1	IMFL2
Total reads	60063910	62401086	57857950	59716094
Total mapped	42879675 (71.39%)	45311164 (72.61%)	41473305 (71.68%)	43052414 (72.1%)
Multiple mapped	1021027 (1.7%)	1029884 (1.65%)	1013360 (1.75%)	1190835 (1.99%)
Uniquely mapped	41858648 (69.69%)	44281280 (70.96%)	40459945 (69.93%)	41861579 (70.1%)
Non-splice reads	24719076 (41.15%)	26119518 (41.86%)	23288965 (40.25%)	25891090 (43.36%)
Splice reads	17139572 (28.54%)	18161762 (29.1%)	17170980 (29.68%)	15970489 (26.74%)

Note. IMFH1and IMFH2 means samples with extremely high phenotypic values for intramuscular fat content; IMFL1and IMFL2 means extremely low phenotypic values, respectively.

3.3 The Identification of DEGs Related to IMF Metabolism

Using the RPKM method, the differential gene expression profile between IMFH and IMFL was examined. In total, 128 genes were detected significantly different between IMFH and IMFL groups. Of these, 34 genes were down regulated while 94 genes were up regulated. Additionally, the volcanic plot of the two comparison groups was displayed in Figure 1. Furthermore, using integrated analysis of RNA-Seq and gene function, the top 20

genes with the highest absolute value of expression in the thigh muscle tissue between IMFH and IMFL are shown in Table 3. Strikingly, the fat associated genes *SCD1*, *FABP4*, and *LFABP* accounted for a significant proportion.

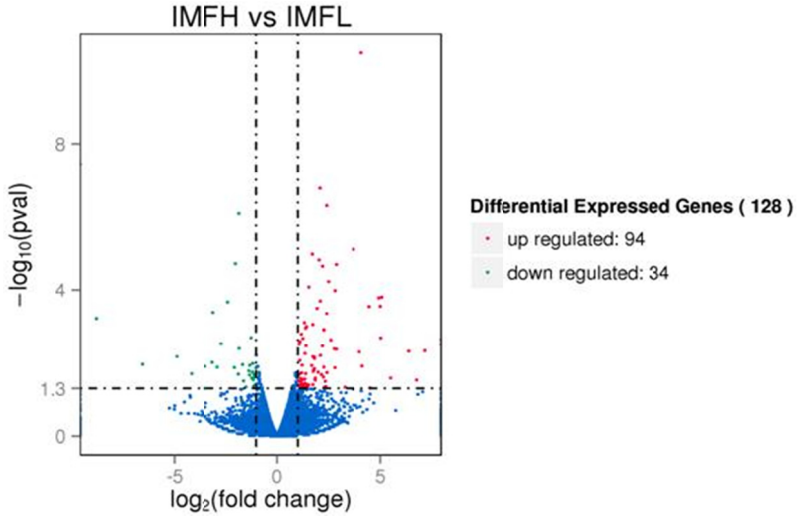

Figure 1. Volcano plot displaying DEGs within two different comparison groups

Note. Significantly DEGs were expressed in red (up-regulated) and green (down-regulated), with no significant difference in genes expressed in blue dots; abscissa representing gene expression fold changes in different samples; and ordinate representing genes statistical significance of differences in expression changes.

Table 3. Top 20 differentially expressed genes between high and low IMF content in thigh muscle tissue

Symbol	Gene name	CHR	Log_2 fold change	P-value	Gene function
SCD1	stearoyl-CoA desaturase	6	1.3369	3.18E-04	involved in fatty acid biosynthesis
FABP4	fatty acid binding protein 4	2	1.5286	4.06E-03	involved in fatty acid uptake, transport, and metabolism
LFABP	fatty acid binding protein 1	4	-1.9926	4.59E-03	metabolism and transport of long-chain fatty acids
SLC1A6	solute carrier family 1, member 6	28	2.0888	3.50E-08	involved in the rapid removal of released glutamate
SLC45A3	solute carrier family 45 member 3	26	1.1902	8.26E-07	related with Glycosaminoglycan metabolism and Metabolism
ACSBG1	acyl-CoA synthetase bubblegum family member 1	10	-1.8164	1.04E-04	plays a central role in brain very long-chain fatty acids metabolism
NALCN	sodium leak channel, non-selective	1	1.3774	6.18E-03	maintenance of substantia nigra pars reticulata
THRSP	thyroid hormone responsive	1	2.3941	6.67E-03	controlling tumor lipid metabolism
PLIN1	perilipin 1	10	2.757	3.06E-03	the inhibition of lipolysis
G0S2	G0/G1 switch 2	26	1.0932	1.15E-02	Regulation of lipid metabolism
SLCO4C1	solute carrier organic anion transporter family member 4C1	Z	1.3165	1.37E-02	the regulation of membrane transport of ouabain
CIDEC	cell death inducing DFFA like effector	11	2.2232	1.52E-02	plays important roles in apoptosis
SS2R	somatostatin receptor 2	18	2.315	2.01E-04	inhibits cell growth
ADORA3	adenosine A3 receptor	26	1.8249	1.91E-05	mediates cell proliferation and cell death
ST8SIA5	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5	Z	1.8304	2.02E-05	involved in the synthesis of gangliosides
LY86	lymphocyte antigen 86	2	1.6957	2.57E-03	mediates the innate immune response
EDN2	endothelin 2	23	-1.0282	5.01E-06	involved in hypertension and ovulation
SNAI2	snail family transcriptional repressor 2	2	1.0259	3.98E-03	involved in the generation of neural crest cells
ST8SIA2	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2	10	-1.0012	1.09E-03	involved in the production of polysialic acid
HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	4	-1.0833	2.12E-03	involved in the metabolism of prostaglandins

3.4 Validation of DEGs

Ten random DEGs (*SCD1*, *SLC1A6*, *FLT3*, *EDN2*, *CCL4*, *RBP7*, *DBX2*, *LY86*, *MSMB*, and *RRP36*) were selected for qRT-PCR to validate the RNA-Seq results and the result showed that the correlations between the mRNA expression level of qRT-PCR and RNA-Seq were all consistent (Figure 2). Thus, the reproducibility and repeatability of gene expression data in this study are reliable.

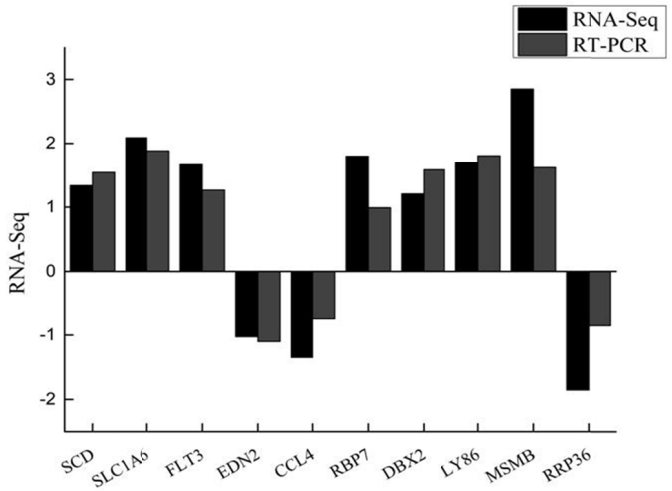

Figure 2. Correlations of mRNA expression level of 10 randomly DEGs between high and low intramuscular fat percentage using RNA-Seq and qRT-PCR

Note. The x-axis represents the gene name, the y-axis represents the log2 (ratio of mRNA levels) measured by RNA-seq and columns of different colors represent data from RT-PCR or RNA-Seq.

3.5 Functional Enrichment Analysis of DEGs

To gain insight into the biological relationships of genes that differentially expressed in thigh muscle tissue between high and low IMF content, we performed GO and KEGG pathway enrichment analysis using Database for Annotation, Visualization and Integrated Discovery (DAVID) bioinformatics resource. The results showed that 12 GO biological process terms related to metabolic process of lipid were significantly enriched ($P < 0.05$), which included steroid biosynthetic process (GO:0006694) and metabolic process (GO:0008202), lipid biosynthetic process (GO:0008610), unsaturated fatty acid metabolic process (GO:0033559), stearoyl-CoA 9-desaturase activity (GO:0004768), acyl-CoA desaturase activity (GO:0016215), unsaturated fatty acid biosynthetic process(GO:0006636), fatty acid metabolic process (GO:0006631), positive regulation of fat cell differentiation (GO:0045600), long-chain fatty acid-CoA ligase activity (GO:0004467), fatty acid derivative metabolic process (GO:1901568) and lipid metabolic process (GO:0006629) (Table 4).

Table 4. Summary of the GO analysis of DEGs

GO accession	Description	No. of DEGs	P-value
GO:0006694	steroid biosynthetic process	4	7.38E-04
GO:0008202	steroid metabolic process	5	9.60E-04
GO:0008610	lipid biosynthetic process	7	3.60E-03
GO:0033559	unsaturated fatty acid metabolic process	3	2.13E-03
GO:0004768	stearoyl-CoA 9-desaturase activity	1	6.27E-03
GO:0016215	acyl-CoA desaturase activity	1	5.31E-03
GO:0006636	unsaturated fatty acid biosynthetic process	2	6.44E-03
GO:0006631	fatty acid metabolic process	4	8.57E-03
GO:0045600	positive regulation of fat cell differentiation	2	2.08E-02
GO:0004467	long-chain fatty acid-CoA ligase activity	1	2.48E-02
GO:1901568	fatty acid derivative metabolic process	2	2.53E-02
GO:0006629	lipid metabolic process	9	3.01E-02

Four KEGG pathways were significantly enriched ($P < 0.05$), including PPAR signaling pathway (gga03320), glycine, serine and threonine metabolism (gga00260), fatty acid metabolism (gga01212) and fatty acid biosynthesis signaling pathway (gga00061).

3.6 Candidate Genes

Integrated analysis of DEGs, GO and KEGG results, QTL mappings and gene function allows us to suggest *FABP4*, *G0S2*, *PLIN1*, *SCD1*, *LFABP*, *SLC1A6*, *SLC45A3*, *ACSBG1*, *LY86*, *ST8SIA5*, *SNAI2*, *HPGD*, *EDN2*, and *THRSP* as the 14 candidate genes for affecting IMF content. Of these, *SNAI2* and *HPGD* involved in steroid biosynthetic and metabolic process, 9 differentially expressed genes (*SCD1*, *ST8SIA2*, *EDN2*, *ACSBG1*, *FABP4*, *G0S2*, *PLIN1*, *THRSP*, and *SLC1A6*) involved in lipid biosynthetic process, unsaturated fatty acid metabolic process and lipid metabolic process. Additionally, two genes, *LFABP* and *SLC45A3*, are important for transport of long-chain fatty acids. Meanwhile, *LY86* positively regulated lipopolysaccharide-mediated signaling pathway. The details of these candidate genes are shown in Table 3. Taken together, the proposed molecular regulatory network affecting IMF metabolism during chicken development is presented in Figure 3. These findings provide new clues for revealing the molecular mechanisms underlying IMF metabolism in chickens.

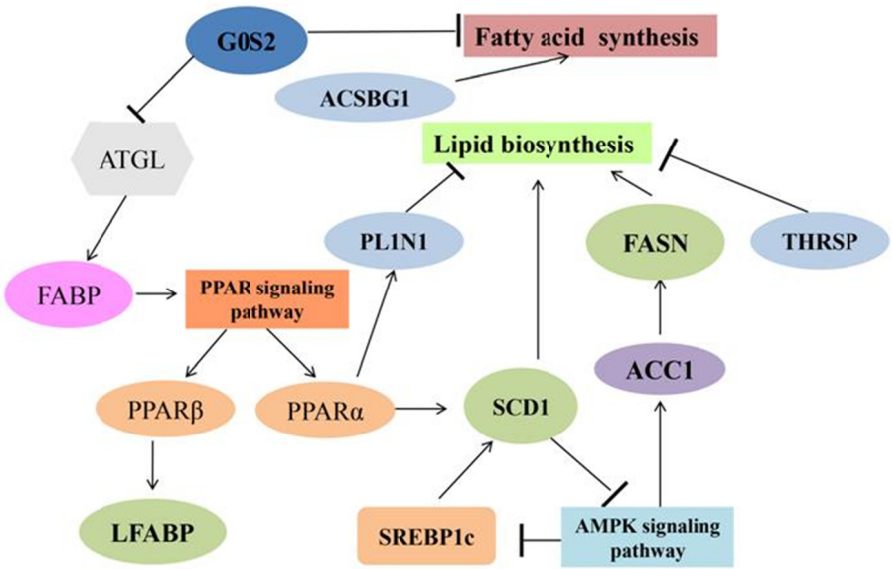

Figure 3. The interaction network of the DEGs between high and low intramuscular fat content.

Note. → represents activate, ⊣ represents inhabit, respectively.

4. Discussion

Fat deposition is highly correlated with meat quality, growth and reproductive performance, and immunity of animals. In chickens, deposited lipids include mainly abdominal fat, subcutaneous fat and IMF. Of these, IMF is an important sensory aspect of meat quality and directly affects the flavor of the meat. Until now, some studies have systematically analyzed the gene expression profiles and regulatory mechanism associated with IMF in breast and thigh tissues of chickens by cDNA microarray. However, few studies have analyzed the thigh tissue transcriptome of chickens using RNA-sequencing. Compared with AA broiler, the Huangshan Black Chicken displayed higher polyunsaturated IMF content in performance traits. Nonetheless, the precise mechanisms of Huangshan Black Chicken contributing to IMF composition remain unclear. Thus, the present study is the first to systematically explore gene expression profiles in thigh tissues using RNA-sequencing to identify global genes and pathways affecting chicken IMF metabolism.

Compared with traditional cDNA microarray technologies, RNA-Seq has many advantages, such as greater dynamic range, removed bias, lower false positives, and higher reproducibility (Cui et al., 2014; Li et al., 2016). Moreover, the correlations between RNA-Seq and the mRNA expression level from qRT-PCR were relatively high (Mao et al., 2015). Obviously, a pooling strategy can dramatically improve accuracy (Kendziorski et al., 2005). In our study, the pooled RNA samples (n = 3 birds) were used for each group and potential candidate DEGs associated with IMF deposition were rigorously defined, with their expression to differ across all

comparisons (IMFH1 vs IMFL1, IMFH1 vs IMFL2, IMFH2 vs IMFL1 and IMFH2 vs IMFL1). To confirm results from the RNA-seq, qRT-PCR was conducted and fold-changes in gene expression between the two methods were correlated ($r^2 = 0.98$) in Huangshan black chickens. Thus, these results showed that RNA-Seq are still recommended to facilitate the accurate detection.

According to integrated analysis on basis of 128 common known DEGs, 14 DEGs (*FABP4*, *G0S2*, *PLIN1*, *SCD1*, *LFABP*, *SLC1A6*, *SLC45A3*, *ACSBG1*, *LY86*, *ST8SIA5*, *SNAI2*, *HPGD*, *EDN2*, and *THRSP*) related to IMF metabolism were detected in this study. Among them, Fatty acid binding protein 4 (*FABP4*) had significantly up-regulated in this study ($P < 0.01$), which was in accordance with that observed in previous reports (Cui et al., 2018). *FABP4* plays an important role in systemic metabolic homeostasis and lipid-mediated biological processes through the regulation of diverse lipid signals (Bag et al., 2015; Floresta et al., 2017). As a lipid chaperon, *FABP4* is responsible for the transportation and metabolism of free fatty acid in adipocyte. Correspondingly, our study revealed that *FABP4* was near to the peak positions of two QTLs for fat traits. These results strongly supported the view of up-regulation of *FABP4* in thigh tissue, revealing that thigh tissue had the stronger lipid biosynthesis.

Metabolic regulation is essential for all biological functions. As a multifaceted regulator, the G0/G1 switch gene 2 (*G0S2*) is abundantly expressed in metabolically active tissues and involved in a variety of cellular functions including proliferation, metabolism, apoptosis and inflammation (Zagani et al., 2015). Particularly, recent studies revealed that *G0S2* acts as a molecular brake on triglyceride (TG) catabolism by selectively inhibiting the activity of rate-limiting lipase adipose triglyceride lipase (ATGL) (Yim et al., 2016; EI-Assaad et al., 2015; Zhang et al., 2017). Similarly, our study revealed that the expression levels of *G0S2* had significantly up-regulated in thigh tissue. In addition, *G0S2* was near to the peak positions of two QTLs for fat traits. In summary, we therefore speculated that *G0S2* may be a promising candidate gene for intramuscular fat percentage in chickens.

As a central regulator of fatty acid metabolism, stearoyl-coenzyme A desaturase 1 (*SCD1*) catalyzes the synthesis of monounsaturated fatty acids (MUFAs), mainly palmitoleate and oleate, which are important in the regulation of lipid and glucose metabolism in metabolic tissues. In addition, *SCD1* is mainly regulated by sterol responsive element binding protein (SREBP)-1c, cyclic AMP response element binding protein 1 (CREB1) and peroxisomal proliferator-activated receptors (PPARs) at the transcriptional level, which were regulatory factors inducing the expression of *SCD1* along with enzymes of denovo fatty acid biosynthesis (ALJohani et al., 2017). Likewise, nucleotide variants of *SCD1* were able to produce significant effects on fatty acid composition, such as milk fat, physicochemical composition, and the quality characteristics in animals (Wen et al., 2018). Among others, the expression levels of *SCD1* had significantly up-regulated in our recent study. Hence, *SCD1* was considered as a major gene affecting fat traits.

Similarly, the expression levels of *THRSP*, *PLIN1*, *SLC1A6*, *SLC45A3*, *ST8SIA5*, *SNAI2*, and *LY86* had significantly up-regulated in thigh tissue ($P < 0.01$). As the previous reported, thyroid hormone responsive (*THRSP*) gene encodes a small acidic protein involved in control of lipogenic enzymes (Liaw & Towle, 1984), perilipin 1 (*PLIN1*) is a lipid droplet-associated protein and has the important function in the regulation of adipocyte lipolysis and lipid storage (Zhou et al., 2016), solute carrier family 1 member 6 (*SLC1A6*) and solute carrier family 1 member 6 (*SLC45A3)* are purported to transport sugars, thereby playing an important potential role in maintaining intracellular glucose levels and the synthesis of long-chain fatty acids (Deng et al., 2007; Shin et al., 2012). ST8 Alpha-N-Acetyl-Neuraminide Alpha-2, 8-Sialyltransferase 5 (*ST8SIA5*) is involved in metabolism and transport of proteins for subsequent modification. However, no previous studies have linked *SNAI2* or *LY86* with lipid differentiation and further study of these genes seems to be warranted.

On the other hand, the expression levels of *LFABP*, *ACSBG1*, *HPGD*, and *EDN2* had significantly down-regulated in our study. Liver-type fatty acid-binding protein (*LFABP*), also frequently known as fatty acid-binding protein 1 (*FABP1*), is involved in intracellular lipid transport from cell membrane to the intracellular sites of fatty acid utilization (Rodriguez et al., 2017). Acyl-CoA Synthetase Bubblegum Family Member 1 (*ACSBG1*) is an acyl-CoA synthetase mediating the activation of long chain fatty acids for the synthesis of cellular lipids, and degradation via beta-oxidation. Additionally, the protein encoded by this gene possesses long-chain acyl-CoA synthetase activity. However, the precise biological functions of *HPGD* and *EDN2* are not known and further research is required to understand the molecular mechanisms of these genes on lipid metabolism in chickens.

Meanwhile, the regulatory network underlying chicken IMF deposition was explored by KEGG pathway analysis. As expected, the well-known PPAR pathway was found and 5 DEGs (novel gene, *PLIN1*, *ACSBG1*,

SCD1, and *LFABP*) involved in PPAR signaling pathway here were screened, which have been proven to be functional in lipid metabolism, such as *PLIN1*, *ACSBG1*, *SCD1*, and *LFABP*. Of special interest, three pathways (fatty acid metabolism, fatty acid biosynthesis and glycine, serine and threonine metabolism) also were enriched, and it was revealed that these three pathways may be the points for the interaction. These findings provide new clues for revealing the molecular mechanisms underlying IMF metabolism in chickens. This novel speculation and its detailed mechanism through pathways related to lipid metabolism identified here needs further validated.

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