Comparison of liver transcriptome from high- and low-intramuscular fat Chaohu ducks provided additional candidate genes for lipid selection

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Abstract

The meat quality of ducks is closely related to the intramuscular fat (IMF) content. This study explored the candidate regulatory genes of IMF formation and lipid deposition in Chaohu ducks. The IMF of breast muscle in 100 ducks was determined and statistically analysed by normal distribution test. Duck liver samples with high IMF (CH, $n = 3$) and low IMF (CL, $n = 3$) were selected for transcriptome analysis by RNA sequencing (RNA-Seq). The IMF was in accordance with normal distribution ($T = 0.001$, $P = 0.999$). The IMF from two tails of the normal distribution was significantly different with $2.9983\% \pm 0.3296\%$ in the CH group and $1.1960\% \pm 0.1481\%$ in the CL group ($P < 0.0001$). RNA-Seq revealed 147 differentially expressed genes, including 78 up-regulated and 69 down-regulated genes in both groups. Validation by qRT-PCR was in agreement with RNA-Seq ($R^2 = 0.838$). Gene ontology analysis revealed that organophosphate catabolism, oxidation–reduction process, cellular lipid catabolism, lipid transport, lipid localisation, lipid biosynthesis and cellular lipid catabolism were involved in lipid metabolism. Meanwhile, Kyoto Encyclopedia of Genes and Genomes pathway analysis suggested that steroid hormone biosynthesis, ovarian steroidogenesis, alpha-linolenic acid metabolism, glycosylphosphatidylinositol anchor biosynthesis and linoleic acid metabolism were involved in lipid deposition, wherein the genes *COMT*, *NT5E*, *PDE4D*, *PLA2G4F*, *A-FABP*, *ADRA2A*, *HSD17B2*, *PPP1R3C*, *PPP1R3B* and *NR0B2* were involved in lipid deposition. This study provided insights into the molecular mechanism for regulating lipid metabolism and identified candidate genes for selecting markers to control IMF formation in Chaohu ducks.

Keywords Chaohu duck · Intramuscular fat · Lipid deposition · Liver transcriptome · Transcriptional regulation

Introduction

Meat quality is closely related to lipid deposition, especially intramuscular fat (IMF). IMF is closely associated with breast muscle lightness, yellowness, cooking loss,

tenderness and flavour, with correlation coefficients of 0.49, 0.47, 0.54, 0.43 and 0.28, respectively, in Pekin duck (Chartrin et al. 2006). Moreover, IMF is influenced by gender, age, feed and genetics (Hocquette et al. 2010; Warner et al. 2010). Its heritability was reported to be 0.36–0.81 in pork and chicken (Chabault et al. 2012; Suzuki et al. 2005) and could be genetically improved by selection (Li et al. 2013; Mortimer et al. 2014). Swine *Myozenin-1* promoter up-regulates swine *peroxisome proliferator-activated receptor-γ2* (*PPAR-γ2*) expression at the RNA and protein levels, which might improve the IMF content (Ma et al. 2015). The gene *perilipin-1* is significantly enriched in the fat metabolism-related PPAR signalling pathway, and its expression level is significantly higher in the high-IMF content group than in the low IMF content group in porcine (Li et al. 2018). Knockdown of the gene *Ubiquitin D* could suppress porcine lipid droplets of intramuscular preadipocyte adipogenesis through the Akt/mTOR signalling pathway (Zhao

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et al. 2018). However, lipid synthesis in poultry is primarily located in the liver and accounts for more than 90%, which is highly distinguished from mammals (Leveille et al. 1968; Zhao et al. 2017). Some studies have investigated the molecular regulatory mechanisms of adipogenesis in ducks. The fat deposition-related pathways, such as MAPK signalling pathway, PPAR signalling pathway, calcium signalling pathway, fat digestion and absorption and TGF- β signalling pathway, are clustered significantly in breast muscle and skin fat in postnatal Pekin duck (Xu et al. 2014). The expression levels of *PPAR- γ* , *C/EBP- α* , *FABP4* and *FAS* genes are gradually increased during preadipocyte differentiation, and *PPAR- γ* knockdown directly reduces lipid production in Jianchang, Cherry Valley and White crested ducks (Ding et al. 2015).

Chaohu duck, an indigenous Chinese meat-type breed, is famous for its superior meat quality and stress resistance. In the present study, Chaohu ducks with different breast IMF contents were selected, and the liver was used for RNA sequencing (RNA-Seq). The transcriptome survey could provide us with additional molecular mechanisms to regulate IMF deposition in Chaohu ducks.

Materials and methods

Animals and sampling

A total of 500 healthy Chaohu duck original species (1 day old, male) were reared under the same environmental conditions from 1 day of age to 21 days of age at the breeding farm in Anhui Yongqiang Poultry Corporation Ltd. (Anqing, China), with feed and water provided ad libitum. At 22 days of age, 100 male ducks were selected by body weight and were divided and raised in indoor separate pens until 12 weeks of age (84 days old). The basic feed formula and nutrient level of all test ducks during the feeding cycle were formulated in accordance with the nutrient requirements of meat-type duck of the Agricultural Industry Standard of the People's Republic of China (Standard number: NY/T 2122-2012). Ducks were slaughtered at 12 weeks of age after 12 h of fasting. Breast muscle was collected immediately and stored at -20°C for IMF detection, and liver samples were stored in liquid nitrogen for RNA extraction.

Measurement of IMF

IMF exists in the outer and inner membranes of the muscle and perimysium. It is evenly distributed in the muscle tissue and closely bound to the membrane protein in the muscle. The IMF of breast muscle was determined by Soxhlet method according to a previous study (Chen et al. 2011). A Soxhlet extraction device (SOX606 fat analyser, Hanon

Instruments Co., Ltd., Jinan, China) was used for fat extraction, and anhydrous ether was used as solvent. The IMF content was expressed as percentage of dry weight.

Total RNA extraction and quality detection

Total RNA from liver samples was extracted using Ezgene™ Biozol RNA Kit (Biomiga Inc., San Diego, USA) according to the manufacturer's guidelines. Dnase I was performed for on-membrane digestion to prevent DNA contamination. The concentration and purity of RNA were detected using a NanoDrop analyser (Thermo Scientific NanoDrop 2000). RNA integrity was detected using the Agilent 2100 Bioanalyzer.

cDNA synthesis, PCR library construction and quality control

The mRNA with polyA was enriched and purified by magnetic beads. mRNA was then broken into 200–300 bp fragments by interrupting ions. The first cDNA strand was synthesised with six random base primers and reverse transcriptase enzyme. The second cDNA strand was synthesised using the first cDNA strand as template, where T was replaced with U. cDNA fragments were then enriched by PCR amplification, and the library was constructed in accordance with snippet size with 300–400 bp bases. The quality was controlled using the Agilent 2100 Bioanalyzer. The total and available concentrations of the cDNA library were controlled by qPCR (Thermo Scientific, StepOnePlus Real-Time PCR System).

RNA-Seq, raw data filter and quality control

The cDNA library was sequenced by paired end with next-generation sequencing. RNA-Seq was performed on an Illumina HiSeq™ 4000 platform by Shanghai Personal Biotechnology Co., Ltd. Raw data of each sample, including sample name, read number, undetermined bases, Q20, Q30 and GC content percentage, were calculated. To filter raw data, cutadapt (version 1.2.1) was used to remove the 3' end joints with at least 10 bp overlap, and a base error rate of less than 20% (Q20) was allowed to create clean reads. Clean reads were then matched to the reference genome GCF_000355885.1_BGI_duck_1.0_genomic.fa ([ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF_000355885.1_BGI_duck_1.0_genomic.fna.gz](ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF_000355885.1_BGI_duck_1.0/GCF_000355885.1_BGI_duck_1.0_genomic.fna.gz)) of *Anas platyrhynchos* by way of Tophat/Tophat2 (Kim and Salzberg 2011).

Gene expression analysis

The count of reads matched to gene was regarded as the expression level of this gene. The expression was normalised

using the following equation and expressed by reads per kilobase million (RPKM):

$$\text{RPKM} = \frac{\text{total exon reads}}{\text{mapped reads (millions)} \times \text{exon length (KB)}}.$$

Following alignment, the normalised RPKM ($\text{RPKM} > 1$) was used as gene expression level for differentially expressed gene (DEG) analysis by DESeq software (version 1.18.0). The DEGs were evaluated by foldchange ($|\log_2\text{foldchange}| > 1$) and statistical significance of difference ($P < 0.05$).

Functional enrichment analysis of DEGs was performed using gene ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (Ashburner et al. 2000; Kanehisa et al. 2007). All DEGs were mapped to each term in the GO database, and the number of DEGs in each term was calculated. Hypergeometric test was used to screen GO terms that were significantly enriched with DEGs when compared with the entire reference genome. The number of DEGs was calculated at different levels in various KEGG pathways. DEGs involved in metabolism and signalling pathways were identified. In the context of the entire genome, a significant enrichment pathway with DEGs was calculated on the basis of the hypergeometric distribution. The analysis of GO terms or KEGG pathways was performed by DAVID 6.8 software (<https://david.ncifcrf.gov/>), with $P < 0.05$ as the threshold value of significant difference.

qRT-PCR analysis

To demonstrate the repeatability and precision of the RNA-Seq gene expression data derived from the Chaohu duck liver libraries, qRT-PCR was performed on 20 DEGs of interest using SYBR Green I Mix with ROX I (Biomiga Inc., San Diego, USA). The 20 pairs of primers were designed online (<http://www.ncbi.nlm.nih.gov/tools/primer-blast/>) (Supplementary Table S1). qRT-PCR was operated using ABI StepOnePlus Real-Time PCR System (ABI 7500, Applied Biosystems, USA). The relative quantitative expression levels of genes were standardised to the internal reference gene β -actin using the $2^{-\Delta\Delta C_t}$ calculation method. The PCR reaction system comprised 25 μL of $2 \times \text{SYBR}$ mixture with ROX I, 1 μL of forward primer (10 μM), 1 μL of reverse primer (10 μM), 2 μL of template DNA and 21 μL RNase-free water, reaching a total volume of 50 μL . A two-step PCR reaction process was employed. The thermal cycling conditions were 95 $^{\circ}\text{C}$ for 10 min, 40 cycles at 95 $^{\circ}\text{C}$ for 15 s and 60 $^{\circ}\text{C}$ for 1 min. Three replicates were set for each amplification. The melt curve analysis programme included 95 $^{\circ}\text{C}$ for 15 s, 60 $^{\circ}\text{C}$ for 1 min, 95 $^{\circ}\text{C}$ for 15 s and 60 $^{\circ}\text{C}$ for

15 s. Cycle threshold (C_t) values were analysed using a linear mixed model (Steibel et al. 2009). The C_t values were converted into quantity using the comparative C_t approach.

Statistical analysis

Univariate analysis (SAS 9.3) was used to analyse IMF to determine its normal distribution, and data from the two tails were compared by paired Student's t test (SAS 9.3). Statistical analysis of genes for validation expression in qRT-PCR was performed by t test (SAS 9.3), and regression analysis between qRT-PCR and RNA-Seq was performed using the Proc Corr method (SAS 9.3). Data were presented as mean $\pm$ standard deviation.

Results

Determination and analysis of IMF in Chaohu ducks

IMF content from breast muscle of Chaohu ducks ranged from 0.83 to 3.74% and exhibited normal distribution ($T = 0.0009$, $P = 0.999$) (Fig. 1a). On the basis of the wide IMF content distribution, three ducks with the highest and lowest contents of IMF were selected and designated as CH and CL groups, respectively, for transcriptomic analysis. The IMF content from the CH group was $2.9983\% \pm 0.3296\%$, and that from the CL group was $1.1960\% \pm 0.1481\%$, showing a significant difference ($P < 0.0001$) (Fig. 1b).

Summary of RNA-Seq data

The average value of raw reads in the six samples was 28,854,834. After quality control of raw reads, 28,786,507 clean reads were obtained in each sample, accounting for 98.29% of raw reads. The Q30 values were 90.82%. The clean data were mapped to the reference genome of *A. platyrhynchos*. The average mapping ratio was 73.33%, in which 93.94% was mapped to the exon (Table 1).

Sequenced data were submitted to the National Center for Biotechnology Information Short Read Archive with accession number of SRP150896 (<https://dataview.ncbi.nlm.nih.gov/?search=SUB4108720>).

DEG analysis

In total, 13,528 genes were detected in the six cDNA libraries (Supplementary Table S2). By taking $P < 0.05$ and $|\log_2\text{foldchange}| > 1$ as the cutoff, a total of 147 DEGs were identified, with 78 up-regulated genes and 69 down-regulated genes in the CH group (Supplementary Table S3,

Fig. 1 Normal distribution and statistical analysis of intra-muscular fat (IMF). **a** Normal distribution curve of IMF in Chaohu ducks, $T=0.000905$ and $P=0.9993$ by univariate (SAS 9.3), dashed vertical axis represents mean of IMF, green shadow showed interval of the low IMF group (CL), red shadow shows interval of the high IMF group (CH). **b** The t test results of IMF indicate a significant difference between the CH and CL group, $P<0.0001$

Table 1 Summary of sequence quality and alignment information from liver transcriptome sequencing of Chaohu ducks

Sample ^a	Reads numbers	Clean reads	Clean ratio (%)	Q30 ^b (%)	Mapped reads ^c (bp)	Mapped ratio (%)	Mapped to exon (%)
CH_1	28,196,550	28,118,162	98.54	90.37	40,632,319	72.26	94.22
CH_2	28,722,276	28,631,650	98.39	90.55	41,497,789	72.47	94.10
CH_3	31,623,857	31,611,980	98.34	90.89	46,699,669	73.86	94.81
CL_1	27,110,273	27,083,164	98.30	92.49	40,648,243	75.05	91.90
CL_2	28,393,304	28,275,141	97.88	89.63	40,768,251	72.09	93.57
CL_3	29,082,744	28,998,947	98.29	91.00	43,042,404	74.22	95.03
Mean	28,854,834	28,786,507	98.29	90.82	42,214,779	73.33	93.94

^aThere are three biological replications in CH and CL group, respectively, with RNA sequencing

^bQ30 is the percentage of bases with recognition accuracy rate more than 99.9%

^cMapped reads are the total number of base pair sequence alignment with the reference genome of *Anas platyrhynchos*

Fig. 2). The DEG expression patterns of each sample were clustered on the basis of the log₂ (fold change) values of their expression ratios, which exhibited good repeatability of samples in the two groups (Fig. 3).

Functional enrichment analysis of DEGs

To further elucidate the functional roles of the 147 DEGs, GO and KEGG pathway enrichment analysis was performed to search for significantly overrepresented

Fig. 2 The Volcano plot of total expression genes of both CH and CL groups. A total of 13,528 genes are expressed in both CH and CL groups, and there are 147 differentially expressed genes (DEGs) by CH vs CL group. The x-axis represents the gene expression fold change values on the log2, the y-axis represents the significance of difference expression P value on the $-\log_{10}$. Vertical bar is twice times difference threshold, horizontal line was $P=0.05$ threshold. Red dots indicate 78 up-regulated DEGs, blue dots indicate 69 down-regulated DEGs and grey dots indicated 13,381 non-differentially expressed genes

categories. GO terms belonged to three categories, namely, biological process, cellular component and molecular function, and 108 terms ($P < 0.05$) were significantly enriched in the three categories (Supplementary Table S4). The top 23 terms (including 20 terms for biological process, 2 terms for cellular component and 1 term for molecular function) were further analysed to determine their regulatory function. Within the biological process, the oxidation–reduction process (GO:0055114) was a considerably broad GO term with 13% candidate genes being annotated within the term, and *HSD17B2*, *PPP1R3C* and *PPP1R3B* genes that participated in lipid metabolism were differentially expressed in this term. GO terms of cellular catabolic process (GO:0044248) with 13% candidate genes were also annotated with the term. Aromatic compound catabolic process (GO: 0019439) had nearly 8.7% candidate genes being annotated with the term. Organic cyclic compound catabolic process (GO:1901361) with 8.7% for cluster frequency, and organonitrogen compound catabolic process (GO:1901565) with 7.2% for cluster frequency, in which three biological process were responsible for lipid metabolism were also significantly annotated (Fig. 4). In addition, the genes *COMT*, *HSD17B2*, *PDE4D*, *PLA2G4F*, *NT5E*, *A-FABP* and *ADRA2A*, which are responsible for fatty acid synthesis or metabolism, were highly enriched in the biological process (Table 2).

KEGG analysis of DEGs

The 147 DEGs were also integrated into the KEGG pathway database (Supplementary Table S5), and a total of 14 pathways ($P < 0.05$) were significantly enriched. The top five significant pathways, including steroid hormone biosynthesis, ovarian steroidogenesis, alpha-linolenic acid metabolism, glycosylphosphatidylinositol (GPI)-anchor biosynthesis (ko00563) and linoleic acid metabolism, were directly related to lipid biosynthesis (Fig. 5). Four genes, *COMT*, *HSD17B2*, *PLA2G4F* and *NT5E*, were highly enriched in GO terms and significantly expressed in KEGG pathways for regulation of fatty acid biosynthesis (Table 2).

qRT-PCR validation

To validate the RNA-Seq results, 20 DEGs, including 10 up-regulated genes (*PER3*, *UNC93A*, *ACADSB*, *TPMT*, *KCNJ16*, *CCND2*, *VWA3B*, *DUSP1*, *LCT*, *PMM1*) and 10 down-regulated genes (*PPP1R3C*, *SIRT5*, *DHCR7*, *ADIPOQ*, *SH3PXD2B*, *KCNJ11*, *SLC50A1*, *NT5E*, *COMT*, *UGP2*), were randomly selected for qRT-PCR analysis (Supplementary Table S6). All the selected DEGs showed concordant expression patterns between the RNA-Seq and qPCR results (Fig. 6a), and the correlation was $R^2 = 0.8383$ (Fig. 6b).

Discussion

IMF content positively influences meat quality, including flavour, juiciness and tenderness in different species (Cui et al. 2012; Van Laack et al. 2001). High IMF content is associated with high meat quality (Chartrin et al. 2006). IMF and polyunsaturated fatty acids are involved in producing intense aroma and flavour of duck meat (Yan et al. 2017). IMF content contributes to flavour and, most importantly, nutrient composition of poultry meat (Matitaputty et al. 2015). This study revealed that the distribution of IMF content within Chaohu ducks showed large variation. The IMF contents in breast and thigh tissues of chickens at the age of 42 and 90 days were detected in a normal distribution (Cui et al. 2018). The IMF content of rabbits displays divergent variation with 0.34–2.7 g/100 g in the longissimus dorsi muscle (Martínez-Álvaro et al. 2017). Thus, the IMF content was mostly regulated from the genetic level.

IMF is different from intermuscular fat, which refers to the fat located between different muscles in the same cut. Thus, further studies are needed to better understand the biological mechanisms of IMF biosynthesis. Biological markers that can predict IMF deposition in advance in farm animals must be identified to satisfy consumers and competitiveness of producers. IMF is positively correlated with abdominal

Fig. 3 Liver expression profiles of 147 differentially expressed genes by CH vs CL group. Colour scale represents RPKM normalised log₁₀ transformed counts. Horizontal bar represents genes. Vertical column represents samples. Red colour indicates higher expression genes, while green colour indicates lower expression genes

fat; hence, breeders usually select a midpoint to increase IMF content and control abdominal fat in a certain low level in poultry (Resnyk et al. 2017). Huang et al. (2016) stated that *LPL* mRNA expression levels in the breast are positively correlated with IMF in Chinese Guangxi san-huang ($r=0.712$, $P=0.001$) and Arbor Acres ($r=0.644$, $P=0.001$) broilers. Chen et al. (2017) also found that the highest levels of *PPAR-γ* are expressed at approximately 150 days in female Wuhua chickens and positively correlated with IMF

($r=0.875$, $P=0.065$). The mRNA expression levels of *ADIPOR1* and *ADIPOR2* genes in the thigh muscle of male Tibetan chickens are significantly positively correlated with IMF (Zhang et al. 2017). The expression of *A-FABP* gene is also positively associated with IMF content in chickens (Wang et al. 2017).

Nuclear receptor small heterodimer partner (encoded by the *NR0B2* gene), which is enriched in the bile secretion pathway and influences the biosynthesis of bile acid through

Fig. 4 GO terms of differentially expressed genes (DEGs) enrichment. There are 23 top terms by GO enrichment. The x-axis represents GO terms, and the y-axis represents the cluster frequency DEGs. Top 23 GO terms include 20 terms for biological process, 2 terms for cellular component, and 1 term for molecular function. Within the 20 biological process, oxidation reduction process (GO:0055114), cellular catabolic process (GO:0044248), aromatic compound catabolic process (GO:0019439), organic cyclic compound catabolic process (GO:1901361) and organonitrogen compound catabolic process (GO:1901565) were responsible for lipid metabolism

Table 2 Information of ten differentially expressed genes associated with lipid metabolism

Gene name ^a	NCBI_ID	Readcount_CH	Readcount_CL	Log2FC ^b	P value	Descriptions
<i>HSD17B2</i>	101792379	1.55	36.50	4.56	3.14E-05	Hydroxysteroid 17-beta dehydrogenase 2
<i>PDE4D</i>	101794939	51.69	105.25	1.03	0.046	Phosphodiesterase 4D
<i>A-FABP</i>	101792626	52.14	112.91	1.11	0.037	Fatty acid-binding protein, adipocyte
<i>NR0B2</i>	101799243	1998.78	932.49	-1.10	0.0010	Nuclear receptor subfamily 0 group B member 2
<i>PPP1R3C</i>	101793994	3588.72	851.91	-2.07	6.59E-09	Protein phosphatase 1 regulatory subunit 3C
<i>PPP1R3B</i>	101796035	926.56	300.39	-1.62	0.0086	Protein phosphatase 1 regulatory subunit 3B
<i>ADRA2A</i>	101803955	18.65	1.04	-4.16	0.0046	Adrenoceptor alpha 2A
<i>NT5E</i>	101794662	5890.18	2931.30	-1.01	0.0022	5'-Nucleotidase ecto
<i>COMT</i>	101805407	36,675.80	14,933.07	-1.30	0.0057	Catechol-O-methyltransferase
<i>PLA2G4F</i>	101794951	50.43	17.96	-1.49	0.036	Phospholipase A2 group IVF

^aThere are ten differentially expressed genes for regulation of lipid biosynthesis highly enriched in GO terms and KEGG pathways ($P < 0.05$)

^bLog2FC is log2FoldChange of readcount by CL group (readcount_CL) vs CH group (readcount_CH), of which three genes up-regulated expression (log2FC > 0) and seven genes down-regulated expression (log2FC < 0)

inhibition of *CYP450* expression, was down-regulated in the CH group, accelerating fat decomposition (Båvner et al. 2005).

The *PPP1R3C* and *PPP1R3B* genes, which encode the protein phosphatase 1 and are enriched in insulin signalling

pathway (ko04910), were down-regulated in the CH group. The protein phosphatase 1 was activated through phosphatidylinositol 3-kinase (PI3K)/Akt signalling and suppressed glycogen synthesis by dephosphorylation in insulin signalling pathway, which participated in fat synthesis (Munro

Fig. 5 KEGG pathways of differentially expressed genes (DEGs) enrichment. There are 20 top pathways by KEGG enrichment. The size of dots indicate the number of expression genes on the pathways, and the colour of dots represent *P* value of the significantly pathway. There are five pathways with significant difference ($P < 0.05$) related to lipid biosynthesis, including steroid hormone biosynthesis (ko00140), ovarian steroidogenesis (ko04913), alpha-linolenic acid metabolism (ko00592), glycosylphosphatidylinositol (GPI)-anchor biosynthesis (ko00563) and linoleic acid metabolism (ko00591)

Fig. 6 Illustration of confirmation results for 20 differentially expressed genes (DEGs) between qRT-PCR and RNA-Seq. **A**. The *x*-axis denotes 20 DEGs name and *y*-axis denotes the log₂Fold-Change derived from RNA-Seq and qRT-PCR, respectively, the mark (*) indicates that gene expression had a significant difference,

$P < 0.05$. **B** The regression analysis between the RNA-Seq (*x*-axis) and qRT-PCR (*y*-axis) with log₂FoldChange values of 20 DEGs, the regression equation: $y = 0.6847x + 0.1115$, with $R^2 = 0.8383$. The round black dots indicate the 20 DEGs

et al. 2005). Glucose starvation regulated the activity of glycogen synthesis by inducing *PPP1Rs* (Carmean et al. 2016). The dephosphorylation of glycogen synthase was catalysed by *PP1* bound to *PPP1R3* (Zois and Harris 2016). Insulin further activated the PI3K/Akt pathway and stimulated glycogen synthesis by inhibiting glycogen synthase kinase-3 and activating *PP1* (Bouskila et al. 2010), which further suggested that the *PPP1R3C* and *PPP1R3B* genes affected glycogen and promoted fatty acid synthesis.

The *PLA2G4F* gene, which was down-regulated in the α -linolenic acid, linoleic acid, arachidonic acid and RAS signalling pathways, was activated via the MAPK pathway and promoted unsaturated fatty acid catabolism by inducing acetyl-CoA dehydrogenase, lipoxygenase and fat dioxygenase (Schaloske and Dennis 2006). The *PLA2X* and *PLA2IVF* genes participated in linoleic acid metabolism, and the *PLA2s* exerted their specific functions by producing lipid mediators, altering membrane phospholipid composition and degrading foreign phospholipids in microorganisms (Murakami et al. 2014; Ducheze et al. 2015). Linoleic acid was positively correlated with abdominal fat pad, which was regulated by *PPAR- γ* mRNA expression in the abdominal adipose tissue (Shokryzadan et al. 2017). The *FABP* gene reduced the accumulation of intracellular free fatty acids by transporting fatty acids to the intracellular part (Ockner et al. 1972). The expression levels of *LPL*, *FABP4* and *FABP3* were significantly down-regulated in the breast, suggesting that *PPAR- γ* and its downstream genes had the essential regulatory function for IMF deposition (Cui et al. 2018; Royan and Navidshad 2016). These findings further suggest that down-regulating *PLA2G4F* could restrain unsaturated fatty acid degradation by inhibiting acetyl-CoA dehydrogenase, lipoxygenase and fat dioxygenase and activating *PPAR- γ* signalling pathway and its downstream genes.

The up-regulation of hydroxysteroid 17- β dehydrogenase 2 (*HSD17B2*) in the steroid hormone biosynthesis pathway and ovarian steroidogenesis pathway regulated the HDL-C, LDL-C and TC levels by promoting testosterone or estradiol generation (Plante et al. 2009). The *COMT* gene, which controls dopamine level, induces tyrosine metabolism and generates L-adrenaline (Bonifacio et al. 2007), was down-regulated in steroid hormone biosynthesis. Lipid metabolism was regulated by one of the membrane-bound transcription factors of *SREBP*, in which *SREBP-1a* was one of the strong activators of all of those genes that mediated the synthesis of cholesterol, fatty acids and triglycerides. *SREBP-1c* activated the transcription of genes required for fatty acid synthesis, whereas *SREBP-2* enhanced cholesterol synthesis (Horton et al. 2002; Otto and Lane 2008). Alpha-2-adrenergic receptor, encoded by *ADRA2A*, was the main presynaptic inhibitory feedback G protein-coupled receptor that participated in regulating norepinephrine release (Link et al. 1992). Compared with the overexpression of a wild-type *ADRA2A*

construct in human embryonic kidney-293 cells and differentiated 3T3-L1 adipocytes, the mutant *ADRA2A* produced more cAMP and glycerol (Garg et al. 2016), which suggested that the activation of *ADRA2A* inhibits cAMP production and reduces lipolysis in adipocytes by the cGMP-PKG signalling pathway.

The phosphodiesterase-4d, encoded by *PDE4D*, resisted fat decomposition by inhibiting the cAMP signalling pathway (Bader et al. 2006) and was down-regulated in the CH group. This finding suggested that *PDE4D* could activate the cAMP signalling pathway, restrain PKA activation and inhibit *PPAR- α* signalling pathway to regulate lipid synthesis. Two non-synonymous SNPs in *NT5E* affected the amount of IMP and its degradation products in beef by regulating the enzymatic activity of *NT5E* (Uemoto et al. 2017). Ecto-5'-nucleotidase (*NT5E*) could convert AMP into adenosine and transform IMP into inosine (Resta et al. 1998), and its down-regulation might suggest a hampered fat degradation that occurred in the CH group.

In summary, the variation pattern of IMF conformed to a normal distribution in Chaohu ducks. Ten genes, *COMT*, *NT5E*, *PDE4D*, *PLA2G4F*, *A-FABP*, *ADRA2A*, *HSD17B2*, *PPP1R3C*, *PPP1R3B* and *NR0B2*, displayed a crucial regulatory role in lipid metabolism and IMF deposition. Through transcriptome analysis, five key regulatory pathways, namely, steroid hormone biosynthesis, ovarian steroidogenesis, alpha-linolenic acid metabolism, GPI anchor biosynthesis and linoleic acid metabolism, played a crucial regulatory role in fat deposition by neural and/or humoral regulation. This study provided insights into the molecular mechanism for regulating lipid metabolism and identified candidate genes for selecting markers to control IMF synthesis in Chaohu ducks.

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Author contributions KG drafted the manuscript and prepared the figures and tables; ZG, XC and KG conceived and designed the experiments and revised them critically for important content; KG, JK and LY performed the experiments; KG and XC analysed the data; JK and LY contributed reagents and materials. All authors approved the final draft of the manuscript submitted for review and publication.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethics All experimental procedures and sample collection were performed according to the Regulations for the Administration of Affairs Concerning Experimental Animals (Ministry of Science and Technology, China; revised in June 2004) and approved by the Institutional Animal Care and Use Committee of Anhui Agricultural University, Hefei, China, under permit no. ZXD-P20140809. This experiment

was performed in accordance with approved relevant guidelines and regulations.

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