Chicken CDS2 isoforms presented distinct spatio-temporal expression pattern and regulated by insulin in a breed-specific manner

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ABSTRACT The cytidine diphosphate diacylglycerol synthases (CDSs) gene encodes the cytidine diphosphate-diacylglycerol (CDP-DAG) synthase enzyme that catalyzes the formation of CDP-diacylglycerol from phosphatidic acid. At present, there are no reports of CDS2 in birds. Here, we identified chicken CDS2 transcripts by combining conventional RT-PCR amplification, 5′ rapid amplification of cDNA ends (RACE), and 3′ RACE, explored the spatio-temporal expression profiles of total CDS2 and the longest transcript variant CDS2-4, and investigated the effect of exogenous insulin on the mRNA level of total CDS2 via quantitative RT-PCR. Four transcripts of chicken CDS2 (CDS2-1, -2, -3, and -4) were identified, which were alternatively spliced at the 3′-untranslated region (UTR). Both total CDS2 and CDS2-4 were prominently expressed in adipose tissue, and exhibited low expression in liver and pectoralis of 49-day-old chickens. Regarding the spatio-temporal expression patterns of CDS2 in chicken, total CDS2 exhibited a similar temporal expression tendency with a high level in the later period of incubation (embryonic day 19 [E19] or 1-day-old) in the brain, liver, and pectoralis. While CDS2-4 presented a distinct temporal expression pattern in these tissues, CDS2-4 levels peaked at 21 d in the brain and pectoralis, while liver CDS2-4 mRNA levels were highest at the early stage of hatching (E10). Total CDS2 (P < 0.001) and CDS2-4 (P = 0.0090) mRNA levels in the liver were differentially regulated throughout the development of the chicken. Total CDS2 levels in the liver of Silky chickens were higher than that of the broiler in the basal state and after insulin stimulation. Exogenous insulin significantly down-regulated the level of total CDS2 at 240 min in the pectoralis of Silky chickens (P < 0.01). In conclusion, chicken CDS2 isoforms with variation at the 3′-UTR were identified, which was prominently expressed in adipose tissue. Total CDS2 and CDS2-4 presented distinct spatio-temporal expression patterns, that is they were differentially regulated with age in brain, liver, and pectoralis. Insulin could regulate chicken CDS2 levels in a breed- and tissue-specific manner.

Key words: chicken, CDS2, transcript variant, gene expression, insulin

INTRODUCTION

Cytidine diphosphate diacylglycerol synthases (CDSs) are critical enzymes that catalyze the synthesis of cytidine diphosphate diacylglycerol (CDP-DAG) from phosphatidic acid to phospholipids such as phosphatidylinositol, phosphatidylglycerol, and cardiolipin (Liu et al., 2014b; Qi et al., 2016a). The gene encoding CDS was first cloned from Escherichia coli in 1985 (Ichio et al., 1985), while the first eukaryotic CDS was cloned from Drosophila in 1995, which shares 31% amino acid similarity with bacterial CDS (Wu et al., 1995). CDS cDNA sequences were subsequently cloned from yeast (Shen et al., 1996), human (Heacock et al., 1996; Lykidis et al., 1997; Weeks et al., 1997; Halford et al., 1998), rat (Saito et al., 1997), mouse (Volta et al., 1999; Inglis-Broadgate et al., 2005), and pig (Mercaide et al., 2007). All eukaryotic genomes have been shown to contain CDS homologs (Lykidis et al., 1997; Kong et al., 2017; Blunsom et al., 2018) and the number of CDS genes varies in different organisms (Blunsom and Cockcroft, 2020). There is one CDS gene in yeast and fly (Liu et al., 2014a), and 2 CDS genes (CDS1 and CDS2) in vertebrates (Lykidis et al., 1997), including rat, mice, human, and zebrafish (Blunsom and Cockcroft, 2020).

The expression of mammalian CDS2 is ubiquitous (Saito et al., 1997; Volta et al., 1999; Inglis-Broadgate et al., 2005). Recent research revealed that CDS is involved in numerous cellular functions. Studies from Drosophila highlight the importance of CDS (CdsA) in the visual photo-transduction system (Wu et al., 1995). Mutations in the eye-specific CDS gene in Drosophila resulted in a defect in photo-
transduction and retinal degeneration (Wu et al., 1995). CDS also plays an important role in the regulation of vascular endothelial growth factor-A (VEGFA) signaling and angiogenesis (Zhao et al., 2019). Loss of CDS2 has been shown to cause a defect in VEGF signaling activity and angiogenic capacity in zebrafish, primarily by decreasing the level of phosphatidylinositol 4,5-bisphosphate (PIP2) regeneration (Pan et al., 2012). Genetic ablation of CDS2 switches the output of VEGFA signaling from promoting angiogenesis to inducing vessel regression and tumor inhibition (Zhao et al., 2019). Recent reports have revealed that the CDS gene is involved in lipid metabolism (Liu et al., 2014b; Qi et al., 2016a; Kong et al., 2017; Qi et al., 2017; Xu et al., 2019), and mammalian CDS1 and CDS2 regulate lipid droplets through distinct mechanisms (Xu et al., 2019). Human CDS1 and CDS2 can create different CDP-DAG pools; CDS2 is selective for acyl chains at the sn-1 and sn-2 positions (D'Souza et al., 2014). The importance of CDS enzymes stems from the need for cells to maintain their phosphoinositide levels, in particular, those of PIP2 enzymes stems from the need for cells to maintain their phosphoinositide levels, in particular, those of PIP2 (Lykidis et al., 1997; Blunsom and Cockcroft, 2020). The translocator assembly and maintenance protein 41 (Tam41) also catalyzes the synthesis of CDP-DAG using phosphatidic acid and cytidine 5′-triphosphate as substrates, it shares no sequence or structural homology with the CDS enzyme (Liu et al., 2014a; Jiao et al., 2019). In addition, CDS is an integral membrane protein, while Tam41 is a peripheral membrane protein (Liu et al., 2014a; Blunson et al., 2018; Jiao et al., 2019).

It was reported that about approximately 92 to 94% of human genes with multiple exons undergo alternative splicing (Wang et al., 2008). Alternative splicing is closely related to the biological processes including cell proliferation, differentiation, and development, and these splicing variants may play different roles (Qi et al., 2016b; Yang et al., 2020). Rapid Amplification of cDNA Ends (RACE) is an efficient way to identify alternatively spliced products if the full-length sequence or even part of a gene is known (Yeku et al., 2009).

Until now, there have been no reports of the CDS gene in birds. In this study, we identified chicken CDS2 transcripts by combining 5′ and 3′-RACE and conventional Reverse Transcription PCR (RT-PCR), analyzed the genomic structure, chromosomal synteny, spatio-temporal expression pattern, and explored the effect of exogenous insulin on chicken CDS2, revealing the basic characteristics of chicken CDS2, the related research would lay a foundation for further identifying the function of chicken CDS2 in the regulation of chicken development and growth. In addition, as an important model animal, the related research on chicken CDS2 would shed a light on the function of human CDS2 from another view.

MATERIALS AND METHODS

Sample Collection

The all procedures carried out was approved by the Animal Care and Use Committee of Henan Agricultural University (approval No. HNND20191201). Chickens and fertilized eggs were obtained from the poultry germplasm resources farm of Henan Agricultural University. The 1-day-old Arbor Acres (AA) broilers and Silky chickens were cage raised routinely until 49 d with free access to food and water as described previously (Ji et al., 2020). The diet was prepared based on nutritional standards for broilers recommended by the NRC (Nutrient Requirements for Poultry, 1994). The fertilized eggs from the Silky population were collected and hatched under conventional conditions.

To identify the spatio-temporal characteristics of chicken CDS2 transcripts, brain, liver, and pectoralis tissues were collected from Silky chicken embryos at E10, E15, E19, and healthy male Silky chickens at 1-day-old (newly hatched birds, D1), D21, and D49 (n = 3 for each time point).

To investigate the effect of exogenous insulin on chicken blood glucose and CDS2 mRNA expression, at 47 d (after 12 h fasting), healthy female AA broilers (average weight, 1.86 kg, n = 28) and female Silky fowls (average weight, 0.75 kg, n = 28) were randomly selected and subjected to an insulin tolerance test with hypodermic injection of 80 μg/kg body weight insulin (n = 20 for each breed) or equal PBS solution as the control (n = 12 for each breed) as described previously (Ji et al., 2020), and birds were given feed at 120 min. In detail, 10 birds of insulin group and 12 birds of PBS group were used to determine the blood glucose concentrations at different time point via the wing vein with a handheld glucometer (ACCU-CHEK Performa, Roche, Germany), followed by 6 birds of insulin group were sacrificed at 0, 120, and 240 min after insulin injection for sampling. The related samples were collected and snap-frozen immediately in liquid nitrogen and transfer to −80°C for RNA isolation.

RNA Extraction and First-Strand Complementary DNA Synthesis

Total RNA was extracted from tissues according to the manufacturer’s instructions (RNAiso Plus, TaKaRa, Dalian, China). The quality and concentration of the extracted RNA was determined by agarose gel electrophoresis and a spectrophotometer (Thermo NanoDrop One, DE), respectively, and then the RNA for RT-PCR was reverse-transcribed using a PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa) in a 20 μL reaction containing 1 μg of total RNA and random primers. The synthesized cDNA samples were stored at −20°C.

Identification of CDS2 Transcripts

CDS2 transcripts were identified by combining conventional RT-PCR amplification and 5′ and 3′ rapid amplification of cDNA ends (RACE). The primers for amplifying the CDS2 gene were designed based on the sequence of EST CR406381.1 and the predicted chicken CDS2 sequence (accession no. XM_417669, XM_004947552
and XM_004947553), and listed in Table S1. CDS2 transcripts were identified from the ovary tissue/mix cDNA of Silky chickens. Specifically, CDS2 transcripts from the ovary tissue of Silky chickens was identify using 5’ and 3’ RACE with the SMART RACE cDNA Amplification Kit (Clontech Laboratories Inc., CA) according to the manufacturer’s instructions. PCR was conducted in a total volume of 50 μL, containing 2.5 μL 5’ or 3’-ready cDNA, 1.0 μL GSP1 or GSP2 primer (10 μM), 2.5 μL UPM (10 μM), and 41.5 μL Master Mix and was performed according to a touchdown PCR protocol: 5 cycles of amplification (94°C for 30 s and 72°C for 3 min) followed by five cycles of 94°C for 30 s, 70°C for 30 s, and 72°C for 3 min, and 25 cycles of 94°C for 30 s, 68°C for 30 s, and 72°C for 3 min. The amplified products for 5’- and 3’-RACE were purified and cloned into the pMD-18T vector (TaKaRa), and transformed into E. coli DH5α. Finally, 6 to 10 positive clones for each band were confirmed by Sanger sequencing. In addition, conventional RT-PCR were used to extend the sequence, products were electrophoresed with 1.5% agarose gel, and conducted sanger sequencing.

**Conventional RT-PCR**

Conventional RT-PCR was conducted to determine the tissue expression profiles of chicken CDS2 transcripts with primers presented in Table S1. The β-actin was used as an internal control. PCR amplification was done in a total volume of 10 μL containing 1.0 μL cDNA, 0.25 μL of each primer (10 μM) and 5 μL 2 × M5 Taq PCR Mix (Mei5bio, Beijing, China). A thermal cycling protocol as follows was used to detect the expression of objective genes: 3 min denaturation at 95°C, followed by 35 cycles of 25 s at 94°C, 25 s at 58 to 60°C, 30 s at 72°C, and the final extension step was 72°C for 5 min, then kept at 4°C forever. Finally, 5 μL PCR products were electrophoresed with 1.5% agarose gel. No template PCR products were taken as the negative control.

**Quantitative Real-Time PCR**

qPCR was carried out to quantity the mRNA expression of total CDS2 and CDS2-4 in samples with a CFX96 Real-Time PCR Detection Systems (Bio-Rad Laboratories, Hercules, CA using 2 × M5 HiPer Dual Real-time PCR Super Mix (SYBRgreen with anti-Taq, Mei5bio). The β-actin gene was used as an internal control to normalize genes’ expression. The primers for qPCR (Table S1) were optimized as previously described (Bustin, 2002). PCR amplification was performed in a total volume of 10 μL containing 5 μL Real-time PCR Super Mix (SYBRgreen with anti-Taq, Mei5bio), 1.0 μL cDNA, and 0.25 μmol/L of forward and reverse primer as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 10 s, 60°C for 15 s, and 72°C for 30 s, and a final incubation at 72°C for 10 min, followed by a final 5 min extension at 72°C. Three technical replicates were performed for each sample. No template PCR amplifications for objective genes were taken as the negative control. Relative gene expression was calculated using the \(2^{-\Delta\Delta CT}\) method (Lykidis et al., 1997). In addition, the data with relatively large variations were re-examined and confirmed by qPCR.

**Bioinformatics Analysis**

CDS2 sequence similarity was compared with the NCBI database (https://www.ncbi.nlm.nih.gov/). The genomic structure of chicken CDS2 was analyzed with the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgBlat). PhyloView in Genomicus v100.01 was used to analyze the consensus conserved genomic synteny of CDS2 (https://www.genomicus.biologie.ens.fr/genomicus-100.01/cgi-bin/search.pl). Amino acid sequence alignment was performed using ClustalW software. A neighbor-joining phylogenetic tree was constructed based on aligned amino acid sequence using MEGA 6.0, and edited by the tree view program with a bootstrap value of 1,000.

**Statistical Analysis**

Data were analyzed by one-way analysis of variance using IBM SPSS Statistics 22.0 software and expressed as mean ± standard error. Multiple comparisons were conducted using the Bonferroni method. GraphPad Prism 5.0 software was used to prepare graphs. \(P < 0.05\) was considered significant and \(P < 0.01\) was considered extremely significant.

**RESULTS**

**Cloning and Identification of Chicken CDS2 Splice Variants**

In the NCBI database, there are three predicted transcripts of chicken CDS2 (XM_417669, XM_004947552 and XM_004947553) with difference in the 5’ region (about 220 bp). Comparing with XM_417669, XM_004947553 had longer predicted 5’-untranslated region (UTR) and an “AGTTGC” deletion in the predicted 5’ coding region, while XM_004947552 had big difference with XM_004947553 and XM_417669 in the 5’ sequence (about 220bp). Based on EST sequence (CR406381.1) and the predicted chicken CDS2 sequence (XM_417669, XM_004947553 and XM_004947552), four CDS2 transcripts alternatively spliced at the 3’-UTR were cloned by combining conventional PCR amplification (LP1-LP5, Figure S1), 5’ RACE (Figure 1A), and 3’ RACE (Figure 1B) and named as CDS2-1 (4,643 bp, GenBank accession no. KC886604), CDS2-2 (4,770 bp, GenBank accession no. KC886602), CDS2-3 (4,893 bp, GenBank accession no. KC886601), and CDS2-4 (5,545 bp, GenBank accession no. KC886603). The gained longest transcript CDS2-4 was consistent with chicken predicted CDS2 nucleotide sequence XM_417669 and XM_004947553. Cloned...
CDS2 transcripts containing part of the 5'-UTR, 1,348 bp coding sequence (same as the predicted coding sequence of XM_417669), and a long 3'-UTR ranged from 3,265 to 4,167 bp with a polyA signal (AATAAA) and polyA tail, with differences at 4,310 to 5,210 bp compared with the longest transcript CDS2-4, and were predicted to encode proteins of 448 amino acids.

Chicken CDS2 is located at chr.22, spanning approximately 23 kb of the genome (Table S2). The longest splice form (CDS2-4) contains 13 exons with the exon-intron boundary (Table S2) abiding by the GT-AG rule; while CDS2-1, CDS2-2, and CDS2-3 contain 14 exons. The exon-intron boundary of intron 13 abides by AC-AG for CDS2-3, GC-CA for CDS2-2, and CT-AG for CDS2-1 (http://genome.ucsc.edu/cgi-bin/hgBlat, Table S2).

**Conservation of CDS2 Sequences Among Species**

We investigated the sequence similarity of the coding sequence, predicted AA sequence, and the 3'-UTR of CDS2 among species. The chicken CDS2 coding sequence shared 96% similarity with turkey (XM_021375090.1), 94% with duck (XM_032204029.1), 81.0% with human (NM_003818.3), 81.2% with mouse (NM_138651.6), 81.1% with cattle (NM_001078046.1), 76.5% with frog (NM_001126503.1), and 78.7% with zebrafish (NM_201186.1). The deduced amino acid sequence of chicken CDS2 shared 99.1% homology with turkey (XP_010721376.1), 97% with duck (XP_032059919.1), 91.4% with human (NP_003809.1), 93.0% with mouse (NP_619592.1), 91.0% with cattle (NP_001071514.1), 85.9% with zebrafish (NP_957480.1), and 87.7% with frog (NP_001119975.1; Figure 2A). The 3'-UTR sequence of chicken CDS2 (KC886603) was also conserved among birds, which shares 87.7% identity with quail (XM_015883014.2), 75.3% with duck (XM_032204029.1), and 75.2% with goose (XM_013199234.1), while no identical sequences were identified in the 3'-UTR between birds and mammals (data not shown).

Syntenic analysis revealed that chicken CDS2 was located between PCNA and ARHGAP25/BMP10 on chr.22, close to the chromosomal breakage/fusion point of the mammalian/bird evolutionary event. The upstream chromosomal region of CDS2 in birds containing the CDS2-PCNA-TMEM230-SVCT gene is homologous to chr.20 in human and chr.2 in mouse while the downstream chromosomal region containing ARHGAP25-BMP10-GKN2-GKN1 was homologous to chr.2 in human and chr.6 in mouse (Figure 3). The amino acid phylogenetic tree of CDS2 (Figure 2B) reflects the evolutionary relationship among species similar to the syntenic analysis of chromosomes (Figure 3).

**Tissue Expression Profile of Chicken CDS2**

We conducted RT-PCR and qPCR to investigate the tissue expression pattern of chicken CDS2 in 49-day-old Silky chickens with AS1, CP2, and QP2 primer sets (Table S1). Primer pair AS1, spanning the alternative splicing region, can simultaneously amplify 4 splicing forms; It showed that CDS2-4 was predominantly expressed in all detected tissues, while the expression of isoforms CDS2-3, CDS2-1, and CDS2-2 was weak as detected by 3'-RACE (Figure 4).

CP2 primer set located in the coding region, whose amplification could reflect the total expression level of CDS2 (referred to as total CDS2). The qPCR revealed that total CDS2 was relatively highly expressed in adipose tissues including sebum (at the tail root), abdominal fat, and neck fat, followed by heart and leg muscle, and weakly expressed in the liver and pectoralis (P < 0.0001, n = 3, Figure 5A).

QP2 primer set located in the specific region of the longest transcript CDS2-4, it could specifically detect the expression of CDS2-4 variant. The qPCR revealed that CDS2-4 had a similar expression change pattern as total CDS2 overall. Whereas compared with the tissue expression pattern of total CDS2 detected with the CP2 primer set (P = 0.0528, n = 3, Figure 5A), the level of CDS2-4 showed greater tissue fluctuation (P < 0.0001, n = 3, Figure 5B). CDS2-4 exhibited similar mRNA levels in heart and adipose tissues including sebum, abdominal fat, and neck fat. CDS2-4 level in abdominal fat was significantly higher than that in other tissues (P < 0.01). The CDS2-4 level in adipose tissues was approximately
4-fold that in the testis and 120-fold that in the liver (Figure 5B).

The Spatio-Temporal Expression Patterns of Total CDS2 and CDS2-4

We further investigated the spatio-temporal expression patterns of total CDS2 (with the CP2 primer set) and CDS2-4 (with the QP2 primer set) by qPCR (n = 3). One-way ANOVA was used to analyze the effect of age and tissue on the expression of total CDS2 and CDS2-4 separately. Total CDS2 exhibited similar temporal expression patterns (Figure 6A) in the brain (P = 0.1623), liver (P < 0.0001), and pectoralis (P = 0.1712). The total CDS2 mRNA level was the highest in the later stage of embryogenesis (embryonic day 19 [E19] or one-day-old [D1]), and the E19 level in the liver was significantly higher than that of the other time points (P < 0.05). In addition, the relative expression of total CDS2 in the brain was significantly (P < 0.05) higher than that in the liver (9-fold) and pectoralis (3-fold) at 49 d (Figure 6A).

Unlike total CDS2, the mRNA level of the CDS2-4 variant presented a distinct temporal change in pattern in the brain, liver, and pectoralis (n = 3, Figure 6B).

Figure 2. Amino acid comparison and phylogenetic analysis of CDS2 gene. (A) Amino acid comparison of the CDS2 gene among species, where highly conservative bases among species were marked with *. (B) Phylogenetic analysis of CDS2 amino acid sequences. The distance scale was presented. The tree was constructed with aligned amino acid sequences by the neighbor-joining method.

Figure 3. Gene arrangement of the genomic region encompassing CDS2. Boxes in the same column with the same color represent homologous genes in the corresponding species. Arrows represent the transcription direction of genes.
CDS2-4 levels peaked at 21 d in the brain \((P = 0.1336)\) and pectoralis \((P = 0.0104)\). The level of CDS2-4 in the pectoralis at 21 d was significantly higher than that at the other ages tested, with the exception of 1 d \((P < 0.05)\); whereas hepatic CDS2-4 levels presented a decreasing trend with development, with highest levels at the early stage of hatching (E10), a weak decrease during embryogenesis (E14−D1), and a clear decrease with age after hatching (D21 and D49, \(P = 0.009\)). Hepatic CDS2-4 levels at E10 were significantly higher than that at 21 d \((P < 0.05)\) and 49 d \((P < 0.01; \text{Figure 6B})\). In addition, the brain had the highest level of CDS2-4 at all time-points among the three tissues; brain CDS2-4 level at 21 d was significantly higher than that in the liver and pectoralis \((P < 0.01, \text{Figure 6B})\).

**Effect of Exogenous Insulin on Chicken CDS2 Expression**

Exogenous insulin resulted to the rapid drop of blood glucose (until 120 min) in both Silky chickens and broilers, and Silky chickens presented a more rapid recovery of blood glucose than broilers after 120 min (Figures 7A and 7B). Exogenous insulin weakly upregulated the mRNA level of total CDS2 at 240 min in the livers of broilers \((P = 0.078, \text{Figure 7C})\); and it downregulated the expression of total CDS2 in the pectoralis of Silky fowl \((P = 0.003, n = 5–6, \text{Figure 7F})\), where the total CDS2 level at 240 min was significantly lower than that at 0 and 120 min (Figure 7F). The mRNA level of total CDS2 in the liver of Silky chickens was higher than that of broilers at the basal state and after insulin stimulation \((P < 0.05, n = 5–6, \text{Figure 7G})\); Whereas the total CDS2 level in the pectoralis of Silky chickens was lower than that of broiler chickens at 240 min after insulin injection \((P < 0.05, n = 5–6, \text{Figure 7H})\).

**DISCUSSION**

RACE technique is an efficient method for identifying the alternative splice variants of genes. In the present study, four isoforms alternatively spliced at the 3′-UTR

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**Figure 4.** Tissue expression pattern of CDS2 detected by RT-PCR with AS1 primer sets. (A) AS1 PCR products; (B) the β-actin gene was used as an internal control. Abbreviations: AF, abdominal fat; BU, bursa of Fabricius; BR, brain; GL, glandular stomach; HE, heart; LI, liver; LE, leg muscle; LU, lung; KI, kidney; M, DNA marker; MU, muscular stomach; PE, pectoralis; PA, pancreas; SE, sebum; SK, skin; SP, spleen.

**Figure 5.** Tissue expression profile of chicken total CDS2 and CDS-4 detected by qPCR. (A) Total CDS2; (B) CDS2-4. The β-actin gene was used as an internal control. Data were analyzed by one-way analysis of variance and expressed as mean ± standard error. Data were analyzed by one-way analysis of variance and expressed as mean ± standard error; pair comparison was conducted using the Bonferroni method with AF as a control. **, \(P < 0.01\); ns, \(P > 0.05\). n = 3. Three technical replicates were performed for each sample. Abbreviations: AF, abdominal fat; BR, brain; BU, bursa of Fabricius; CA, cartilage; GL, glandular stomach; HE, heart; LE, leg muscle; LI, liver; LU, lung; KI, kidney; M, DNA marker (DL 700); MU, muscular stomach; NE, negative control; NE, neck fat; PA, pancreas; PE, pectoralis; OV, ovary; SK, skin; SE, sebum; SP, spleen; TE, testis.
were identified from birds' CDS2. It is well known that different isoforms of a single gene function differently. Transcripts expressed in low level may have important function too, which could be turned up when they are required (Pope and Medzhitov, 2018). CDS2-4 isoform and total CDS2 showed distinct spatio-temporal expression patterns, which suggested that the other CDS2 isoforms (isoform 1–3) detected to be expressed in low level here should present dynamic expression in different tissues and development stages, and each isoform may function in its own manner. 3’-UTRs are major players in gene regulation that enable local function (Ciolli Mattioli et al., 2019), compartmentalization, and cooperativity (Mayr, 2017). Birds and mammals share high sequence similarity for the deduced CDS2 proteins, while possessing their own conservative 3’-UTR, suggesting that the 3’-UTR of CDS2 may play an important role in regulating the phenotypic diversity of higher organisms (Mayr, 2017). The average length of 3’-UTRs in human are 1,278 nt (Zhao et al., 2011); CDS2 exhibited a long 3’-UTR both in mammals (9,000 nt in human) and birds (5,210 nt in chicken), suggesting there may be an abundance of regulatory elements in the 3’-UTR of CDS2. The shortening/lengthening of the 3’-UTR may result in a change in miRNA binding sites (Yuan et al., 2019). Further works are needed to identify the separate function of these isoforms and the interaction between miRNAs and chicken CDS2.

Here, we observed that chicken CDS2 was predominantly expressed in multiple adipose tissues, which provide direct evidence for the function of CDS2 in birds’ lipid metabolism. CDS genes in mammals have been linked with lipid metabolism (Liu et al., 2014b; Qi et al., 2016a, 2017; Kong et al., 2017; Xu et al., 2019), with a
Figure 7. Effect of insulin on blood glucose and total CDS2 mRNA levels. (A) Blood glucose in PBS control. (B) Blood glucose after insulin treatment (80 \( \mu \)g/kg body weight). (C) Total CDS2 in liver of AA broilers. (D) Total CDS2 in liver of Silky chickens. (E) Total CDS2 in pectoralis of broilers. (F) Total CDS2 in pectoralis of Silky chickens. (G) Total CDS2 in liver. (H) Total CDS2 in pectoralis. C–F, different letters indicate \( P < 0.05 \), no letter or same letter indicates \( P > 0.05 \). In G and H, data were only compared between two breeds at the same time-point. *, \( P < 0.05 \); ns, \( P > 0.05 \). Data were analyzed by one-way analysis of variance and presented as mean ± standard error. All detected samples were included. n = 5–6. Three technical replicates were performed for each sample.
stronger effect on phosphatidic acid levels. CDS2 deficiency (while not CDS1) could impair the maturation of initial lipid droplets in cultured mammalian cells (Xu et al., 2019). A transcriptome profile revealed that CDS2 was differentially expressed in adipose tissues of fat- and short-tailed Chinese sheep (Wang et al., 2014).

Livers in birds play a central role in lipid metabolism, serving as the center for lipoprotein uptake, formation, and export to the circulation (Arvind et al., 2000; Alshamy et al., 2019), while adipose tissue functions primarily as a storage tissue (Na et al., 2018). In this study, the level of CDS2 was low in AA chicken livers at 49 d, but where mRNA levels of CDS2 showed dramatic temporal changes. Zhao et al. reported that hepatic triglyceride levels increased during embryonic development (Gomez and Rodriguez, 1987; Zhao et al., 2007). While post-hatching, the liver lipid content correlated negatively with broiler age; it was the highest on the first day then decreased sharply at d 7 (Noble and Ogungyemi, 1989; Alshamy et al., 2019). Here, the exhibited dynamic change pattern of total CDS2 matched well with that of hepatic lipids during development and growth of birds, which implied that CDS2 may participate in the regulation of hepatic lipid metabolism. In addition, total CDS2 and CDS2-4 present distinct temporal change feature, which indicated different CDS2 transcripts in birds may function in a different way.

Here, we found that total CDS2 levels in livers of Silky chickens were twice that of broilers at 47 d. Meanwhile, it has been reported that the serum lipid level (total cholesterol and triglyceride) of Silky chickens was higher than that of broilers (Ji et al., 2020), which also suggested that hepatic CDS2 contribute to the bird’s lipid metabolism. In addition, the previous data from our group found that a 30% energy restriction in broiler chickens significantly reduced the abdominal fat ratio and subcutaneous fat thickness (Chen et al., 2012), and downregulated an unknown differentially expressed fragment C7-2 (mapped to chicken EST CR406381.1; Wang et al., 2012) in broiler’s liver tissue. With the update of the chicken genomic database in NCBI, this fragment has now been mapped to the 3'-UTR of chicken CDS2 (XM_004947553, XM_417669, and XM_004947552). The downregulation of CDS2 in chicken liver by energy restriction suggested the association of chicken CDS2 with fat metabolism.

CDS2 was reported to be prominently expressed in brain of murine (Yue et al., 2014) and human (Fagerberg et al., 2014), here we also observed the main transcript CDS2-4 in birds highly expressed in the brain throughout the embryonic development and growth stage (comparing with liver and pectoralis tissues), which suggested the conservative function of CDS2 in brain among species. The structure and function of brain also depend on intercellular exchange and de novo synthesis of lipids. Cell communication in the brain involves constant lipid exchange through lipoproteins, microvesicles, and non-esterified fatty acids (Loving and Bruce, 2020; Chausse et al., 2021).

CDS2 in Drosophila has been linked with insulin pathway (Liu et al., 2014b). Here we further study the insulin sensitivity of birds’ CDS2. Silky chickens showed greater rise latency of blood glucose than broilers at 240 min after insulin stimulation. Skeletal muscles have been considered to be a major regulator of systemic glucose homeostasis (Honka et al., 2018). In the current study, exogenous insulin significantly downregulated the level of total CDS2 at 240 min in the pectoralis of Silky chickens, which means the birds’ CDS2 response to exogenous insulin in a breed- and tissue-specific manner. The downregulation of CDS2 in the pectoralis of Silky may contribute to the more rapid blood glucose recovery at 240 min after insulin injection through positively downregulating the PI3K activity in the insulin pathway (Liu et al., 2014b).

Overall, chicken CDS2 were alternatively spliced at 3'-UTR, which prominently expressed in adipose tissues and exhibited distinct spatio-temporal expression patterns during chicken development and growth. Total CDS2 mRNA level in the liver of Silky chickens was twice of that in broilers. Total CDS2 in pectoralis was insulin sensitive, which were significantly changed by insulin in a breed- and tissue-specific manner. Nevertheless, limited to the experiment design, monosexual birds, and limited samples were used in this study, so the impact of these factors on the findings/conclusions we made from these experiments need to be considered.

CONCLUSIONS

In summary, 4 alternatively spliced forms of CDS2 with variations at the 3'-UTR were identified in chicken, and the longest form, CDS2-4, was the main transcript. Both total CDS2 and CDS2-4 were extensively expressed in all detected tissues, and predominantly present in adipose tissues including sebum, abdominal fat, and neck fat. Furthermore, total CDS2 and CDS2-4 presented distinct spatio-temporal expression patterns in the brain, liver, and pectoralis during chicken development and growth. Total hepatic CDS2 and CDS2-4 were dramatically regulated with age in a distinctly different manner. Total CDS2 in the liver of Silky chickens was significantly higher than that of broilers at the basal state and after insulin stimulation. Total CDS2 mRNA level was changed by insulin in a breed- and tissue-specific manner, which was significantly downregulated at 240 min after exogenous insulin stimulation in the pectoralis of Silky chickens.

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DISCLOSURES

The authors declare that they have no competing interests.
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