

## cDNA cloning and expression analysis of *ATGL* gene in four avian species\*

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Adipose triglyceride lipase (*ATGL*) is a new key triglyceride-specific lipase that participates in the lipolysis in adipose tissue. The full cDNA of *ATGL* gene in Chinese francolin, pigeon, bengalese-finch and house sparrow was cloned to reveal its tissue-specific expression by mRNA real time analysis. The obtained cDNA of chinese francolin *ATGL* gene (*cfATGL*) was 1465 bp long, and contained 13 bp 5'-untranslated region (5'UTR) and 1452 bp open reading frame (ORF) encoding a 483-amino acid peptide. All the obtained cDNA of pigeon *ATGL* gene (*pATGL*), as well as that of bengalese-finch (*bfATGL*) and house sparrow (*hsATGL*) was 1459 bp long, including 13 bp 5'UTR and 1446 bp ORF encoding 481 amino acids. The identities of *ATGL* gene among these birds occurred no less than 88.4% by homology analysis. As indicated by mRNA real time analysis in Chinese francolin tissues, *ATGL* gene was predominantly expressed in leg muscle, heart and breast muscles of birds of both sexes. In pigeons, *ATGL* gene was shown to be predominantly expressed in abdominal fat, subcutaneous fat and breast muscle in males, and in subcutaneous fat, leg muscle, heart and abdominal fat in females. In bengalese-finch, very high *ATGL* mRNA level was found in subcutaneous, heart, breast muscle, abdominal and leg muscle fat in males, and in breast muscle, leg muscle, abdominal and subcutaneous fat in females. In house sparrow, higher *ATGL* mRNA level was detected in subcutaneous, breast muscle, leg muscle and abdominal fat in males, and in breast

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muscle, heart and leg muscle fat in females. In conclusion, the *ATGL* cDNA of Chinese francolin, pigeon, bengalless-finch and house sparrow was obtained and predominantly expressed in adipose, muscle and heart tissues.

**KEY WORDS:** *ATGL* gene / bengalless-finch / cDNA / chinese francolin / expression / house sparrow / pigeon

Adipose tissue triglyceride is the main form of energy storage, affecting energy balance *via* its synthesis and mobilization. In 2004, three independently working teams found adipose triglyceride lipase (ATGL) to hydrolyze triglycerides [Jenkins *et al.* 2004, Villena *et al.* 2004, Zimmermann *et al.* 2004]. The mice with *ATGL* knocked out gene were proved to display lipolysis [Haemmerle *et al.* 2006]. It is now known that ATGL in mammals is a rate-limiting enzyme, catalyzing the initial step of triglyceride hydrolysis.

ATGL cooperated with hormone sensitive lipase (HSL) to consume the stored adipose in adipose and nonadipose tissues [Zechner *et al.* 2009]. TGL catalyzes the first step of triglyceride hydrolysis to generate diglycerides (DG) and free fatty acids (FA) – Zimmermann *et al.* [2004]. DGs are subsequently degraded to monoglycerides (MG), and further to glycerol and FA by HSL [Haemmerle *et al.* 2002]. When the requirement for energy increases, FAs are secreted directly into blood to provide energy for other tissues. The pattern of *ATGL* gene expression affects the amount of fat deposit. *ATGL* deficiency in mice resulted in obesity and accumulation of excess fat in heart, which finally led to cardiac dysfunction and premature death [Haemmerle *et al.* 2006]. ATGL was also showed to have transacylase and phospholipase activity, thus participating in the synthesis of triglycerides [Lake *et al.* 2005, Haemmerle *et al.* 2006]. This function of ATGL in triglyceride hydrolysis and synthesis indicates that it plays a vital role in the energy dynamic balance.

ATGL activity requires the activation of CGI-58 also named Abhydrolase Domain-Containing 5 (ABHD5). Therefore, ATGL is a CGI-58-activated triglyceride hydrolase [Lass *et al.* 2006]. CGI-58 is distributed predominantly on the surface of lipid droplets (LDs) and plays a crucial role in TG degradation in cells [Yamaguchi *et al.* 2001]. Although the mechanism is still unknown, ATGL interacts with CGI-58, and then increases TG hydrolase activity up to 20-fold. Variations in *ATGL* and *CGI-58* gene were found related to systemic TG accumulation [Schweiger *et al.* 2009].

Recently, G<sub>o</sub>/G<sub>1</sub> switch gene 2 (*G0S2*) was identified as inhibitor of ATGL. *G0S2* specifically interacts with ATGL, inhibits ATGL lipase activity and attenuates ATGL-mediated lipolysis [Yang *et al.* 2010]. ATGL hydrolyzes triglycerides by interacting with both – *G0S2* and CGI-58. CGI-58 stimulates hydrolysis, whereas *G0S2* reduces the co-activation of ATGL with CGI-58 [Brasaemle *et al.* 2010]. CGI-58 and *G0S2* regulate ATGL by non-competing mechanisms [Lu *et al.* 2010].

Both the murine and human *ATGL* genes comprise nine exons and eight introns, and encode two 86% homology proteins of 486 and 506 amino acids, respectively [Jenkins *et al.* 2004, Villena *et al.* 2004, Zimmermann *et al.* 2004]. As a member of

patatin-like phospholipases (PNPLAs) family the ATGL or PNPLA2 proteins contain a patatin domain of three-layer sandwich structure ( $\alpha/\beta/\alpha$ ) that is located in the N-terminal. The catalytic site of ATGL is located in the patatin domain, and consists of Ser-47 within a GX SXG motif and Asp-166 outside the central-sheet [Lake *et al.* 2005, Lass *et al.* 2006, Schweiger *et al.* 2009].

In recent years, as an important candidate gene that influences fat deposition and fleshy traits of animals, *ATGL* gene attracted much attention in pigs, cattle and chickens. The porcine *ATGL* gene played a crucial role in catecholamine-induced lipolysis and was related to carcass traits by Deuliis *et al.* [2008] and Li *et al.* [2010]. The bovine *ATGL* gene was also cloned, and highly expressed in fat, rumen and muscle tissue (skeletal and cardiac) at mRNA level, but solely in the adipose tissue at the protein level [Deuliis *et al.* 2010, Cui *et al.* 2010]. *ATGL* genes in chickens and other avian species were also reported to have unique patterns, *i.e.* although the chicken *ATGL* gene encodes a shorter peptide, it is much longer than in mammals [Saarela *et al.* 2008, Lee *et al.* 2009, Nie *et al.* 2009].

This study aimed at further characterizing the *ATGL* gene in birds by cDNA cloning and mRNA real time analysis using four avian species – Chinese francolin, pigeon, bengalless-finch and house sparrow.

## **Material and methods**

### **Animals and tissues**

One male and one female Chinese francolin were purchased from Guangzhou Changban market (Guangzhou, China). One male and one female pigeon were purchased from Zhaoqing Beilaide Economic Development Co., Ltd. (Zhaoqing, China). Two young bengalless-finches (one male and one female) and two young house sparrows (one male and one female) were purchased from Guangzhou Fangcun Huadiwan Flower Bird Fish & Insect market (Guangzhou, China).

The birds were killed by decapitation to collect samples of abdominal fat (Abd), breast muscle (Brm), cerebrum (Cer), cerebellum (Ceb), gizzard (Giz), heart (Hea), hypothalamus(Hyp), kidney (Kid), leg muscle (Lem), liver (Liv), lung (Lun), pituitary (Pit), subcutaneous fat (Sbf), small intestine (Smi), spleen (Spl), *stomachus glandularis* (Stg), testis (Tes) and ovary (Ova). All samples were quick-frozen in liquid N and then stored at -80°C for long term preservation.

### **Primers**

Thirteen pairs of primers (PM1 to PM13) were used (Tab. 1). Primers PNPLA2F | PNPLA2R | ATGL02F and ATGL02R were designed according to the mRNA sequences of chicken *ATGL* gene (NCBI accession number EU240627), while ZZ5'CDS3F according to the sequence of parrot (NCBI accession number GQ221784). Primers JM5RACE2, MQ3RACE3 and ZG5RACE2 were designed according to obtained partial sequence of bengalless-finch, house sparrow and Chinese francolin.

In the latter, primer PM8 was designed for real time RT-PCR analysis on target gene and primer PM9 was for chicken beta-actin gene ( $\beta$ -actin) – NCBI accession number L08165 – used as internal control. Similar to Chinese francolin, quantification of *ATGL* mRNA real time was also completed in pigeon (PM10, PM11), bengalese (PM12, PM13), and house sparrow (PM12, PM13). PM11 was designed based on pigeon beta-actin gene ( $\beta$ -actin) – NCBI accession number DQ022673 – whereas PM12 and PM13 were based on the conservative region of *ATGL* gene and  $\beta$ -actin gene among chicken, sparrow and zebra finch (NCBI accession numbers L08165, AF416454, AY045726, respectively). All primers were designed using the GeneTool Lite Launcher software (<http://www.BioTools.com/>) and synthesized by BIOSUNE Co. Ltd (Shanghai, China).

#### **cDNA preparation**

Total RNA from all tissues of the four species were isolated by Trizol reagent (TAKARA, Japan) following the manufacturer's instructions. Then using ReverTra Ace- $\alpha$ -<sup>®</sup> reagents (TOYOBO Life Science, Japan), the cDNA was obtained to amplify *ATGL* gene of each bird. The total RNA of each tissue was reversely transcribed with the use of PrimeScript<sup>®</sup> RT reagent Kit With gDNA Eraser (TAKARA, Japan) for real time RT-PCR analysis.

#### **RT-PCR, cloning and sequencing**

The total cDNA from breast muscle tissue was used as template to amplify the *ATGL* cDNA by RT-PCR procedure. All required reagents were mixed according to the instructions given in TaqMix Kit (Dongsheng BIO Co. Ltd, Guangzhou, China). RT-PCR was run in a Bio-Rad thermal cyclers (Bio-Rad Laboratories, CA, USA) at 94°C pre-denaturing for 3 min, followed by 33 cycles of 94°C for 30 s, X°C, annealing for 30 s (Tab. 1), 72°C for 1 min, and final extension of 10 min at 72°C. PCR products were identified with DNA electrophoresis in 1.5% agarose gel and purified with D2500-01 reaction kit (OMEGA Bio-tek, GA, USA). Then target DNA was cloned into PMD18-T vector (TAKARA, Otsu, Japan) and transferred into JM109 competent cells to duplicate, selected the monoclonal bacilli and sent for sequencing by INVITROGEN (Shanghai, China).

#### ***ATGL* gene homology analysis in birds**

Eleven birds (duck, chicken, turkey, quail, parrot, zebra finch, Bengalese finch, house sparrow, Java sparrow, pigeon, Chinese francolin) were selected for *ATGL* gene homology and phylogenetic analysis, the accession numbers of CDS and proteins being available from NCBI (<http://www.ncbi.nlm.nih.gov/>) – Table. 2. DNASTAR soft package (<http://www.dnastar.com>) was used to predict the evolutionary relationship and calculate the identity per cents among species. The comparison of *ATGL* amino acid sequences was completed with the OMEGA 4.1 Beta 2 programme (<http://www.megasoftware.net/mega41.html>).

## The avian ATGL gene

**Table 1.** Thirteen primer pairs used in the study

Primer name	Forward & reverse	Sequence(5' to 3')	Annealing temperature (°C)	Gene <sup>a</sup>	Purpose
PM1	ATGL02F PNPLA2R	gctggtcctctccttgcaatag tcagaagagtggcaggcactc	54	<i>cfATGL</i> , <i>bfATGL</i> , <i>hsATGL</i>	Partial CDS
PM2	ATGL02F ATGL02R	gctggtcctctccttgcaatag gcctggctgaaagatgactgc	56	<i>pATGL</i>	Partial CDS
PM3	PNPLA2F PNPLA2R	atgttcctttggactccgc tcagaagagtggcaggcactc	61	<i>cfATGL</i>	ORF
PM4	ZZ5'CDS3F PNPLA2R	gggtgcggaggccatggt tcagaagagtggcaggcactc	58	<i>pATGL</i>	ORF and partial 5'UTR
PM5	ZZ5'CDS3F JM5RACE2	gggtgcggaggccatggt tgggaggcaagtgttcaaatgatgt	58	<i>bfATGL</i>	Partial CDS and 5'UTR
PM6	ZZ5'CDS3F MQ3RACE3	gggtgcggaggccatggt tgcctcccaactgaaccaagctc	58	<i>hsATGL</i>	The first half CDS and partial 5'UTR
PM7	ZZ5'CDS3F ZG5RACE2	gggtgcggaggccatggt aggcgttagaggttgcgaagg	58	<i>cfATGL</i>	Partial CDS and 5'UTR
PM8	ZGATGLF ZGATGLR	ttgccacgatatgagctgaagaac ggcgatgaagggtgcagga	61	<i>cfATGL</i>	Real time RT-PCR
PM9	ZG β-actin-F ZG β-actin-R	tggcattgctgacaggat ctcctgctgatccacat	61	chinese francolin β-actin	Internal control
PM10	GZATGLF GZATGLR	ccaaaactgaaccaagctctt gctcctcatcactcaatc	61	<i>pATGL</i>	Real time RT-PCR
PM11	GZ β-actin-F GZ β-actin-R	ccccctgctgtcttc tctcatgctatcccattg	61	Pigeon β-actin	Internal control
PM12	ATGL2F ATGL2R	gccccaatagagctgaagaac gccctgctgcacataacc	62	<i>bfATGL</i> , <i>hsATGL</i>	Real time RT-PCR
PM13	βactin2F βactin2R	tgggtatggagctcctgtggtatc ggtgccaggcctgtgatc	62	Bengalese and house sparrow β-actin	Internal control

<sup>a</sup>*cfATGL* – chinese francolin *ATGL* gene; *pATGL* – pigeon *ATGL* gene; *bfATGL* – bengalese-finch *ATGL* gene; *hsATGL* – house sparrow *ATGL* gene.

**Table 2.** CDs and protein databases of *ATGL* used for homology analysis

Species	Latin Name	CDs No.*	Protein No.*
Duck	<i>Anas platyrhynchos</i>	EU747707	ACE80204
Chicken	<i>Gallus gallus</i>	EU852334	ACF60601
Turkey	<i>Meleagris gallopavo</i>	EU852335	ACF60602
Quail	<i>Coturnix japonica</i>	GQ221783	ACT09361
Parrot	<i>Melopsittacus undulatus</i>	GQ221784	ACT09362
Zebra finch	<i>Taeniopygia guttata</i>	HQ260023 <sup>&amp;</sup>	ADP24695 <sup>&amp;</sup>
Bengalese finch	<i>Lonchura striata</i>	HQ260017 <sup>&amp;</sup>	ADP24689 <sup>&amp;</sup>
House sparrow	<i>Passer domesticus</i>	HQ260019 <sup>&amp;</sup>	ADP24691 <sup>&amp;</sup>
Java sparrow	<i>Padda oryzivora</i>	HQ260020 <sup>&amp;</sup>	ADP24692 <sup>&amp;</sup>
Pigeon	<i>Columba livia</i>	HQ260022 <sup>&amp;</sup>	ADP24694 <sup>&amp;</sup>
Chinese francolin	<i>Francolinus pintadeanus</i>	HQ260018 <sup>&amp;</sup>	ADP24690 <sup>&amp;</sup>

\*Referred to NCBI accession numbers. <sup>&</sup> These sequences with dispatched accession numbers were accepted but not released by NCBI website (<http://www.ncbi.nlm.nih.gov/>).

### RT-PCR analysis of *cfATGL*, *pATGL*, *bfATGL* and *hsATGL* gene

The  $\beta$ -actin was used as internal control and SYBR green as fluorescent dye to quantify mRNA level of *cfATGL*, *pATGL*, *bfATGL* and *hsATGL* genes by RT-PCR analysis. Each sample was examined three times to obtain the mean. The reaction mixture and running programme followed the instruction of QPS-201 Realtime PCR reagent (TOYOBO, Japan). Running programme was performed in a Stratagene Mx3005P (STRATAGENE, La Jolla, CA, USA) and sample values were displayed when running was over. Each sample  $\Delta$ Ct was obtained from difference between Ct of *ATGL* and  $\beta$ -actin gene ( $\Delta$ Ct = Ct<sub>target gene</sub> - Ct <sub>$\beta$ -actin</sub>). Then relative expression of each sample was assessed ( $2^{-\Delta$ Ct}).

## Results and discussion

### cDNA sequence of *cfATGL*, *pATGL*, *bfATGL* and *hsATGL* encoding genes

The cDNA of *cfATGL* gene was 1465 bp in length, including 1452 bp ORF (encoding 483 amino acids) and 13 bp incomplete 5'UTR (Fig. 1). The *pATGL* cDNA was 1459 bp long, including 1446 bp ORF (encoding 481 amino acids) and 13 bp incomplete 5'UTR (Fig. 2). The *bfATGL* cDNA was 1459 bp long, including 1446 bp ORF (encoding 481 amino acids) and 13 bp incomplete 5'UTR (Fig. 3). The *hsATGL* cDNA was 1459 bp long, including 1446 bp ORF (encoding 481 amino acids) and 13 bp incomplete 5'UTR (Fig. 4).

### Homology and phylogenetic relationship of ATGL among birds

Among 11 avian species (Tab. 2) the identities of ATGL sequences are very high (over 88.4%). This is presented in Table 3 which shows that the identity among *Galliformes* was higher than 93.1%. The homology among *Passeriformes* reached up to 96.7%. Both pigeon and parrot presented a relatively lower homology with both *Galliformes* and *Passeriformes* (less than 93.3% and 91.2% each).

The constructed phylogenetic tree displayed three distinct groups based on ATGL sequences of birds of 11 species (Fig. 5). The *Galliformes* (chicken, turkey, chinese francolin, quail and duck gathered in one group. Parrot, a species of *Psittaciformes* was a separate group. Pigeon (*Columbiformes*) was grouped with the *Passeriformes* (house sparrow, zebra finch, bengalese-finch and Java sparrow).

### Tissue-specific expression of *cfATGL* gene

In male Chinese francolin, high *ATGL* mRNA level was found in leg muscle (2.4425 $\pm$ 0.6010), heart (1.8921 $\pm$ 0.5076) and breast muscle (0.8370 $\pm$ 0.3041). Medium mRNA level occurred in liver (0.2649 $\pm$ 0.2757), and little or no *ATGL* mRNA was found in the remaining tissues. In female, it was high in leg muscle (4.5683 $\pm$ 0.1273), breast muscle (3.8106 $\pm$ 0.3554) and heart (2.4680 $\pm$ 0.0808). Lower level was identified

The avian ATGL gene

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gggtgaggagccATGTTCCCTTGGACTCCGCTTGAACATCTCGTTCGCCGCTCGCGTTTCCATGAGGGTTACCATATCGCCGTC
M F P L D S A W N I S F A G C G F L G U Y V H I G U
CCGAGCTGCCTCCAGAACATGCCCTTTCCTCGTCCCAACGCCCGAAGGTGTACGGGGCTCGCCGGCCGGCTGACCCACCCGCCC
A S C L Q E H A P F L U A N A R K U V Y G A S A G A L T A T A
CTGCTCAGCCGCGCTCCCTCGGTGAGGCTGGTCCAGCATATTCCAGTCTCAAGAAGCCCAAGAGAGTTCTGGTCCGCTTCCAC
L U S G A C L G E A G A S I I R U S K E A R K R F L G P L H
CCGCTTTCAAGTTCGTGAAGATCATTCCGCTCCGCTCCCAAGACTTCCAGAGATGGCATGAGCTGGCAGCAGAGCATTGGGT
P S F N L U K I I R L C L S K T U P E N G H E U A A G R L G
ATTTCCTCAGCCGAGTGTCTGATGGAAACCATCTACTCTCAGACTTCCATTCAAGAGAGACTGATCCAGGCTCCGCTCCGAGC
I S L T R U S D G E N U I L S D F H S K E E L I Q A C I C S
ACCTTATCTCTACTCTGCTGATCCGCCAAGCTTCCGTCGCTGAGATATTTGATGAGGAATTTCTGCAAGTTTCCACGA
T F I P U V C G L I P P T L R G U R V Y U D G G I S D N L P R
TATGACTGAGAACCAATCACGGTGTCTCCCTTCTCAGGGGAGTGTATCTGCCAGCAGACACTCCCAAGACTGCACAGCTG
V E L K N T I T U S P F S G E S D I C P R D S S T N H E L
AGAGTCACCATAGAACATCCAGTTCACCTTCGCAAGCTTACCGCTCTCAAGGCCCTCTTCTCCAGAGCCACAGGTGTCGGC
R U T N T S I Q F N L R N L Y R L S K A L F P P E P Q U L R
GATATGTCAGAGCCGCTTCCGATCGACTGCACTTCCGAGAGAGATGGTCTCCTGCACTTCATCGCCCAAGCTTCTGCTGCT
D H C K Q G Y R D A L H F L K K N G L L H L H R P S P A G P
CTCCTGCAATAGAGCCCTCCAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
L L A I E A P P G E K K E E E K E V E D Q H E D N T A L A R
GTTGAGAGCAGCATTTCGACACTTCCCTCCAACTGAACAGACTCTTTGGAGGCTTGTCTGAAGAGAGAGAGAGAGAGAGAGAG
U E D H I F E H L P P K L N Q A L L E A C A E R R G L L T G
ATTGAGACAGCCTGATACCTGCTGAGCAGTGCATGTTTCCCTACCTCCCTGCTGAGTGTGCTGTTTCTGCTGAGG
I S N T L P I R U A T A M H U P Y L L P L E S A U S F T U R
TTACTGCAATGCTTCAGATATCCCTGAGGATATGATGATGAGGAGCAGATAGTGAATTCGCAATATCTGTCGAAGAAGCC
L L E W L P D I P E D I R W H R E Q I T E I C N Y L U K K A
AAGAAAGAACTCGGCAGTCTCTTTCAGCCAGGCTTTACTATCAGCTTGCAGTGGAGGCCAGAGCCTCCCAATTTCTCCAGCACT
K K K L G S H L S A R L Y V H L E L G G P Q S L P I S P A S
CCTTGGCCAGAGCAATCCATCTGATGAGAGCAAGCCGCTCCCTGCTGATGTCATGTCAGTGCAGTGCAGGAGAGTCCAGCCAT
P C G E A H P H W H R S N R S L S D U H L K W E E Y Q R Q L
GTGATGGCTTACTCTGATCAGCTGAGCATGAGCAGTCCCTTCTCCCTGGAGAGGATTCAGATAAAGCTTCCAGCTTTGAGACTG
U H G L L C I N U D M Q A S L F P W E G F Q I K L P P L D C
GCAAGAGAGTCCCTGCCACTCTCTGA
A K E C L P L F .
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Fig. 1. cDNA sequence and encoded amino acids of *cfATGL* gene. Capital letters show open reading frame (ORF) and lower case letters show 5'-untranslated region (5'-UTR). Capitals below ORF indicate amino acids of ATGL, and "." refers to the stop codon. "GCGFLG" and "GASAG" in box display two feature structures of lipase ("GXGXXG" and "GXSXG" motifs). Underlined are amino acids of patatin domain.

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gggtgaggagccATGTTCCCGCCGATTCACCTGGAATATCTCCTTCCGCCGCTCGCGTTTCCATGAGGGTTACCATATCGCCGTC
M F P P D S T W N I S F A G C G F L G U Y V H U G U
CCGAGCTGCCTCCAGAACATGCCCTTTCCTCGTCCCAACGCCCGAAGGTGTACGGGGCTCGCCGGCCGGCTGACCCACCCGCCC
A S C L Q E H A P F L U A N A R K U V Y G A S A G A L T A T A
CTGCTCAGCCGCGCTCCCTCGGTGAGGCTGGTCCAGCATATTCCAGTCTCAAGAAGCCCAAGAGAGTTCTGGTCCGCTTCCAC
L U S G A C L G E A G A S I I R U S K E A R K R F L G P L H
CCGCTTTCAAGTTCGTGAAGATCATTCCGCTCCGCTCCCAAGACTTCCAGAGATGGCATGAGCTGGCAGCAGAGCATTGGGT
P S F N L U K I I R M C L S K T U P D N G H E U A A G R L G
ATTTCCTCAGCCGAGTGTCTGATGGAAACCATCTACTCTCAGACTTCCATTCAAGAGAGAGCTGATCCAGGCTCCGCTTCCAGC
I S L T R U S D G E N U I L S D F H S K E E L I Q A C I C S
ACCTTATCTCTACTCTGCTGATCCGCCAAGCTTCCGTCGCTGAGATATTTGATGAGGAATTTCTGCAAGTTTCCACGA
T F I P U V C G L I P P T L R G U R V Y U D G G I S D N L P R
TATGACTGAGAACCAATCACGGTGTCTCCCTTCTCAGGGGAGTGTATCTGCCAGCAGACACTCCCAAGACTGCACAGCTG
V E L K N T I T U S P F S G E S D I C P R D G S T N H E L
AGAGTCACCATAGAACATCCAGTTCACCTTCGTAAGCTTACCGCTCTCAAGGCCCTCTTCTCCAGAGCCACAGGTGTCGGC
R U T N T S I Q F N L R N L Y R L S K A L F P P E P H U L R
GATATGTCAGAGCCGCTTCCGATCGACTGCACTTCCGAGAGAGATGGTCTCCTTCATCCAGTCCPCCGCTTCTCTCCCTT
D H C K Q G Y R D A L H F L K K N G L L H P P S P A R S L L
GCCATAGAGCCCTCCAGCAGATAGGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG
A I E A P A G D K E E E E T E A E D Q L D D N T A L A U V E
GAGCAGTCTTTCAGCATTTCCTCCAACTGAACAGACTCTTTCGAGGCTTGTCTGAAGAGAGAGAGATCTTCTGACTATATCC
E H I F E H L P P K L N Q A L L E A C A E R R S I L T D I T
AGCATGCTCCATAGCTGTCGACCGCAATGATGTCCTTATGCTGCTGAGTCTGCAAGTTTCTTCACTGCTGAGTTCGTCG
N H L P I R U A T A M H U P Y H L P L E S A U S F T U R L L
GAATGCTCCAGATATCCCTGAGGATATGATGATGAGGAGCAGATGACTGAATTCAGACTACCTTCTGAGAGAGCCAGAGAG
E V L P D I P E D I R W H R E Q I T E I C N Y L U K K A R
AACTTGTAGCAGCTTTCAGCCAGCTCTACTTACCTGCACTTGCAGAGCCAGAGAGCTCCCAATTTCTTCCGATGAGTCTT
K L G S H L S A R L Y V H L E L G E P K S L P I S S A L A S
CCTAAGCAGTCTGATGATAGAGCAAGCCCTCCCTATGATGTTATGATGAGTGCAGGAGAGTCCAGCCAGCTTCCGCTG
G E A L P H W I R S N R S L S D U H H K W E E Y Q R Q L L
GCCTTACTGATCAGCTGAGCATGAGCAGCTCCCTTCTCCCTGGAGAGGATTCAGATAAAGCTTCCAGCTTCCAGCTTTCAGCA
G L L C I N U D M Q A S L F P R E G F Q I K L P P L D C A R
GAGTCCCTGCCACTCTCTGA
E C L P L F .
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Fig. 2. cDNA sequence and encoded amino acids of *pATGL* gene. Capital letters show open reading frame (ORF) and lower case letters show 5'-untranslated region (5'-UTR). Capitals below ORF indicate amino acids of ATGL, and "." refers to the stop codon. "GCGFLG" and "GASAG" in box display two feature structures of lipase ("GXGXXG" and "GXSXG" motifs). Underlined are amino acids of patatin domain.

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gggtgaggagccatggtccccctggactccacctgcaatatctccttcgcccggctcgggcttctgggggtgtaccacgtcggcgta
      M F P L D S T W N I S F A G C G F L G U Y H U G U
GCCAGCTGCTGCAGAACATGCCCGTTCCTGGTGCACCACCCAGAGAGGTGTACGGCGCTCGCCGGGGCGCTCACCGCCACCCCA
A S C L Q E H A P F L U A N A K K U Y G A S A G A L T A T A
CTCGTCAGCGCGCTGCTCGGTGAAGCTGGTGCACGATTATTCGGGTGCAAAAGAGCCCGAAGAGGTTCTTGGCCCTCTCCAC
L U S G A C L G E A G A S I I R U S K E A R K R F L G P L H
CCCTCTTTCAGATGGTGAACCATTCGGATGTCCTGTCCAAGTGGTGCACAGAAATGGGCATGAGTTGCAGCAGGAGCTTTGGGT
P S F N L U K T I R H S L S K U U P E N G H E U A A G R L G
ATTCCCTGACACGGCTCTGTGAGGAAATGTGACTGTGACAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGTTCAGT
I S L T R U S D G E N U I L S D F N S K E E L I Q A C I C S
ACCTTTATCCTGTCTACTGTGGCTGATACCTCCAACCTTGCCTGGAGTGAATATGTTGATGGAGGATTTTGCACATTTGCCCAA
T F I P U Y C G L I P P T L R G U R Y U D G G I S D N L P Q
TATGAGTGAAGAACACATACAGGTGTCTCCGTTCTCGGAGAGAGCGATATGCCACCGGACAGTTCACAAATATGATGAGCTG
Y E L K N T I T U S P F S G E S D I C P R D S S T N M H E L
AGAGTCACCAATACAGCATCAGTTCACCTTCGCAACCTTACCAGCTTCAAGGCGCTGTTCTCGGAGCCACAGGTGTCGGG
R U T N T S I Q F N L R N L Y R L S K A L F P P E P Q U L R
GATATGTCAGCAGGCGTATCAGATGCACCTTCCGAGAGAAATGGTCTCCTTCATCCTCAAGTCCCTCGCCCTCTCCTT
D M C K Q G Y R D A L H F L K R N G L L H R P S P A R P L L
CCCATAGAACCTCCAGGAGACAGAGAGGAGAGAACAGAGCTGAGGACCACTAGAGGACCAACCTCCCTTGTGTTGTGA
A I E A P P G E K E E E E T E A E D Q L E D N T A L A U V E
GAGCATATCTTGAACACTTGCCTCCAGACTGAACCAAGCTCTCCTGGAGCTTGTGCTGAAGAGAAAGTTCTTGCATGCTGAGC
E H I F E H L P P R L N Q A L L E A C A E R R S F L T G L S
AACATGTCCTGTGCTGTCGCGACAGCCATGATGCTCCCTACATGCTGCTGAGAGTTCGAGTTTCTTCACTGTCAAGTTGCTC
N M L P U R U A T A H M U P Y M L P L E S A U S F T U R L L
CAATGCTCCAGATATCCCTGAACATATAGATGATGAGGAGCAGATGACAAATCTGCACTATCTTGTGAAGAAAGCCAGAGG
E W L P D I P E D I R W M R E Q M T E I C N Y L U K K A K K
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K L G S H L S A R L Y Y H L E L G G P Q K L P I S P A P C
GGTAGGCACTCCCATGTCGATGAGGACCAACCCCTCCGTTTCTGATGTCATGATGAGTGGGAGGATACCGCCAGCAGCTGCTG
G E A L P M W H R S N R S U S D U M H K W E E Y Q R Q L M L
GGCTGCTGTCATCAAGCTGACATGCAAGCTCCCTCTCCCTCGGAGAGGTTTCAAGTAAAGCTTCCACCTTACAGCTGTGCAAAA
G L L C I N U D M H Q A S L F P R E G F Q L K L P P L D C A K
GAGTCCCTGCCACTTCTCA
E C L P L F .

```

Fig. 3. cDNA sequence and encoded amino acids of *bfATGL* gene. Capital letters show open reading frame (ORF) and lower case letters show 5'-untranslated region (5'-UTR). Capitals below ORF indicate amino acids of ATGL, while “.” refers to the stop codon. “GCGFLG” and “GASAG” in box display two feature structures of lipase (“GXGXXG” and “GX SXG” motifs). Underlined are amino acids of patatin domain.

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gggtgaggagccatggtccccctggactccacctgcaatatctccttcgcccggctcgggcttctgggggtgtaccacgtcggcgta
      M F P L D S T W N I S F A G C G F L G U Y H U G U
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A S C L Q E H A P F L U A N A K K U Y G A S A G A L T A T A
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L U S G A C L G E A G A S I I R A S K E A R K R F L G P L H
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P S F N M U K T I R H S U S K M U P D H G H E U A A G R L G
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I S L T R U S D G E N U I L S D F N S K E E L I Q A C I C S
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T F I P U Y C G L I P P T L R G U R Y U D G G I S D N L P Q
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R U T N T S I Q F N L H N L Y R L S K A L F P P E P Q U L R
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D M C K Q G Y R D A L H F L K R N G L L H R P S P A R P L L
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A I E A P P G E K E E E E T E A E D Q L E D N T A L A U V E
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E H I F E H L P P R L N Q A L L E A C A E R R S F L T G L S
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N M L P U R U A T A H M U P Y M L P L E S A U S F T U R L L
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E W L P D I P E D I R W M R E Q M T E I C S Y L U K K A K K
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K L G S H L S A R L Y Y H L E L G G P Q K L P I S P A P C
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G E A L P M W H R S H R S U S D U M H K W E E Y Q R Q L M L
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G L L C I N U D M H Q A S L F P R E G F Q L K L P P L D C A K
GAGTCCCTGCCACTTCTCA
E C L P L F .

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Fig. 4. cDNA sequence and encoded amino acids of *hsATGL* gene. Capital letters show open reading frame (ORF) and lower case letters show 5'-untranslated region (5'-UTR). Capitals below ORF indicate amino acids of ATGL, while “.” refers to the stop codon. “GCGFLG” and “GASAG” in box display two feature structures of lipase (“GXGXXG” and “GX SXG” motifs). Underlined are amino acids of patatin domain.



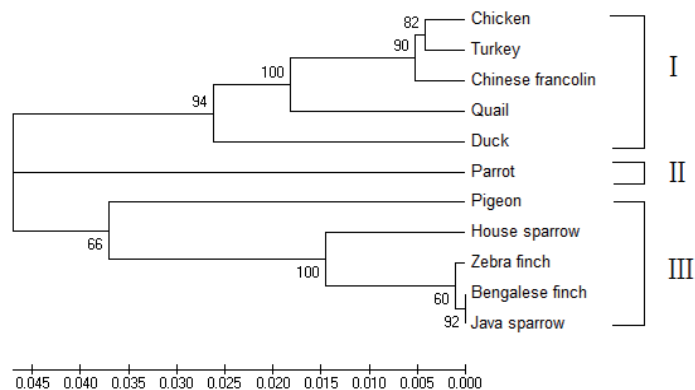


Fig. 5. Phylogenetic tree among 11 bird species based on *ATGL* sequencing. Numbers below branches show identities between adjacent species. The scale line at the bottom of the Figure indicates the mean genetic distance.

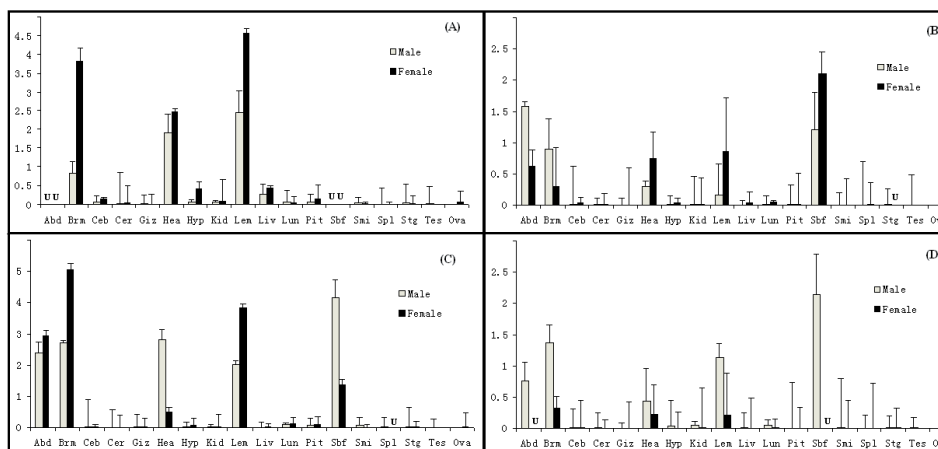


Fig. 6. mRNA level of *ATGL* gene in different tissues of birds. The horizontal axis indicates different tissues whereas vertical axis indicated  $2^{-\Delta C_t}$  value (mean $\pm$ S.D) each, SD – standard deviation. (A) Chinese francolin. (B) pigeon. (C) Bengalese finch. (D) house sparrow. Abbreviations composed of three letters indicate different tissues: abdominal fat (Abd), breast muscle (Brn), cerebellum (Ceb), cerebellum (Ceb), gizzard (Giz), heart (Hea), hypothalamus(Hyp), kidney (Kid), leg muscle (Lem), liver (Liv), lung (Lun), pituitary (Pit), subcutaneous fat (Sbf), small intestine (Smi), spleen (Spl), *stomachus glandularis* (Stg), testis (Tes) and ovary (Ova). “U” represents uncollected tissues.

in liver ( $0.2649\pm 0.2757$ ) and hypothalamus ( $0.4244\pm 0.1644$ ), and little or no *ATGL* mRNA was found in the remaining tissues (Fig. 6 and Tab. 4).

#### Tissue-specific expression of *pATGL* gene

In male pigeon, high *ATGL* mRNA level was found in abdominal fat ( $1.5746\pm 0.0778$ ), subcutaneous fat ( $1.2016\pm 0.5940$ ) and breast muscle

**Table 3.** ATGL cDNA identity among birds of 11 species

Species	Bengalless-finch (%)	Chicken (%)	Chinese francolin (%)	Duck (%)	House sparrow (%)	Java sparrow (%)	Parrot (%)	Pigeon (%)	Quail (%)	Turkey (%)	Zebra finch (%)
Bengalless-finch	92.1										
Chicken		98.6									
Chinese francolin			95.4								
Duck				91.4							
House sparrow					96.7						
Java sparrow						90.2					
Parrot							89.6				
Pigeon								89.4			
Quail									95.4		
Turkey										89.8	
Zebra finch											91.5

**Table 4.** mRNA level of *cfATGL* gene by real time analysis

Tissue	ATGL mRNA of male chinese franclin	ATGL mRNA of female chinese franclin
Abd	failure	failure
Brm	0.8370	3.8106
Ceb	0.0566	0.1504
Cer	0.0130	0.0478
Giz	0.0270	0.0100
Hea	1.8921	2.4680
Hyp	0.0764	0.4244
Kid	0.0730	0.0773
Lem	2.4425	4.5683
Liv	0.2649	0.4263
Lun	0.0615	0.0367
Pit	0.0597	0.1507
Sbf	failure	failure
Smi	0.0356	0.0167
Spl	0.0109	0.0085
Stg	0.0398	0.0120
Tes	0.0140	
Ova		0.0595

Abbreviations composed of three letters indicate different tissues: abdominal fat (Abd), breast muscle (Brm), cerebrum (Cer), cerebellum (Ceb), gizzard (Giz), heart (Hea), hypothalamus (Hyp), kidney (Kid), leg muscle (Lem), liver (Liv), lung (Lun), pituitary (Pit), subcutaneous fat (Sbf), small intestine (Smi), spleen (Spl), stomachus glandularis (Stg), testis (Tes) and ovary (Ova).

**Table 5.** mRNA level of *pATGL* gene by real time analysis

Tissue	<i>ATGL</i> mRNA of male	<i>ATGL</i> mRNA of female
	pigeon	pigeon
Abd	1.5746	0.6099
Brm	0.8961	0.2859
Ceb	0.0183	0.0206
Cer	0.0093	0.0166
Giz	0.0002	0.0004
Hea	0.2849	0.7405
Hyp	0.0145	0.0276
Kid	0.0101	0.0072
Lem	0.1546	0.8507
Liv	0.0026	0.0241
Lun	0.0068	0.0360
Pit	0.0116	0.0085
Sbf	1.2016	2.0994
Smi	0.0014	0.0014
Spl	0.0012	0.0144
Stg	0.0138	failure
Tes	0.0040	
Ova		0.0129

Abbreviations composed of three letters indicate different tissues: abdominal fat (Abd), breast muscle (Brm), cerebrum (Cer), cerebellum (Ceb), gizzard (Giz), heart (Hea), hypothalamus (Hyp), kidney (Kid), leg muscle (Lem), liver (Liv), lung (Lun), pituitary (Pit), subcutaneous fat (Sbf), small intestine (Smi), spleen (Spl), *stomachus glandularis* (Stg), testis (Tes) and ovary (Ova).

(0.8961±0.4808), and medium in heart (0.2849±0.0990) and leg muscle (0.1546±0.4989). Little or no *ATGL* mRNA was found in the remaining tissues. In female, it was high in subcutaneous fat (2.0994±0.3470), leg muscle (0.8507±0.8627), heart (0.7405±0.4219), and abdominal fat (0.6099±0.2695). Lower level was found in breast muscle (0.2859±0.6311), and little or no *ATGL* mRNA was detected in the remaining tissues (Fig. 6 and Tab. 5).

#### Tissue-specific expression of *bfATGL* gene

In male bengalese-finch, high *ATGL* mRNA level was detected in subcutaneous fat (4.1506±0.5742), heart (2.8154±0.3024), breast muscle (2.6945±0.0849), abdominal fat (2.3839±0.3313) and leg muscle (2.0139±0.1058). Little or no *ATGL* mRNA was found in the remaining tissues. In female, it was high in breast muscle (5.0513±0.1795), leg muscle (3.8326±0.1202), abdominal fat (2.9383±0.1414) and subcutaneous fat (1.3692±0.1893). Lower level was found in heart (0.4897±0.1400), whereas little or

**Table 6.** mRNA level of *bfATGL* gene by real time analysis

Tissue	<i>ATGL</i> mRNA of male	<i>ATGL</i> mRNA of female
	bengalese-finch	bengalese-finch
Abd	2.3839	2.9383
Brm	2.6945	5.0513
Ceb	0.0377	0.0288
Cer	0.0100	0.0077
Giz	0.0179	0.0174
Hea	2.8154	0.4897
Hyp	0.0310	0.0409
Kid	0.0354	0.0203
Lem	2.0139	3.8326
Liv	0.0101	0.0153
Lun	0.0752	0.1158
Pit	0.0520	0.0656
Sbf	4.1506	1.3692
Smi	0.0396	0.0086
Spl	0.0139	failure
Stg	0.0290	0.0133
Tes	0.0113	
Ova		0.0241

Abbreviations composed of three letters indicate different tissues: abdominal fat (Abd), breast muscle (Brm), cerebrum (Cer), cerebellum (Ceb), gizzard (Giz), heart (Hea), hypothalamus (Hyp), kidney (Kid), leg muscle (Lem), liver (Liv), lung (Lun), pituitary (Pit), subcutaneous fat (Sbf), small intestine (Smi), spleen (Spl), *stomachus glandularis* (Stg), testis (Tes) and ovary (Ova).

**Table 7.** mRNA level of *hsATGL* gene by real time analysis

Tissue	<i>ATGL</i> mRNA of male house sparrow	<i>ATGL</i> mRNA of female house sparrow
Abd	0.7596	failure
Brm	1.3660	0.3143
Ceb	0.0194	0.0069
Cer	0.0096	0.0029
Giz	0.0031	0.0014
Hea	0.4323	0.2207
Hyp	0.0198	0.0061
Kid	0.0340	0.0105
Lem	1.1251	0.2112
Liv	0.0164	0.0048
Lun	0.0367	0.0151
Pit	0.0061	0.0062
Sbf	2.1386	failure
Smi	0.0106	0.0042
Spl	0.0036	0.0025
Stg	0.0157	0.0163
Tes	0.0089	
Ova		failure

Abbreviations composed of three letters indicate different tissues: abdominal fat (Abd), breast muscle (Brm), cerebrum (Cer), cerebellum (Ceb), gizzard (Giz), heart (Hea), hypothalamus (Hyp), kidney (Kid), leg muscle (Lem), liver (Liv), lung (Lun), pituitary (Pit), subcutaneous fat (Sbf), small intestine (Smi), spleen (Spl), *stomachus glandularis* (Stg), testis (Tes) and ovary (Ova).

by rapid-amplification of cDNA ends (RACE) technology, we failed ultimately to obtain the complete UTR regions. The obtained cDNA of *cfATGL*, *pATGL*, *bfATGL* and *hsATGL* genes all contained 13 bp 5'UTR and the full-length open reading frame (ORF). Their NCBI accession numbers were HQ260018, HQ260022, HQ260017 and HQ260019, respectively. There was a deletion of six nucleotides (CTTCAT) coding for two amino acids (L and H) in *pATGL*, *bfATGL* and *hsATGL* cDNA compared to that of *cfATGL*, and a deletion of six nucleotides (TTCAGC) encoding two amino acids (L and Q) compared to that of chicken [Nie *et al.* 2010]. According to the reported studies, due to frameshift variations, several truncated ATGL peptides were identified in humans [Fischer *et al.* 2007, Nie *et al.* 2010]. In avian species, several nucleotide substitutions were found between two sequences of quail *ATGL* gene [Nie *et al.*, 2010].

ATGL protein patatin and hydrophobic domains are conserved in avian and mammalian species. In this study, based on homology analysis, also ATGL were found conserved among the 11 avian species. Both the active serine hydrolase motif ("GASAG" for "GX SXG") and the glycine rich motif ("GCGFLG" for "GXGXG") were found identical among birds and mammals (humans, pigs, cattle, mouse, and rat) – Akiyama *et al.* [2007], Nie *et al.* [2009], Serr *et al.* [2009]. All avian species in this study had a similar length in amino acid residues ( $481 \pm 2$ , Tab. 2), and a homology

no *ATGL* mRNA was identified in the remaining tissues (Fig. 6 and Tab. 6).

#### Tissue-specific expression of *hsATGL* gene

In male house sparrow, high *ATGL* mRNA level was detected in subcutaneous fat ( $2.1386 \pm 0.6493$ ), breast muscle ( $1.3660 \pm 0.2869$ ), leg muscle ( $1.1251 \pm 0.2193$ ) and abdominal fat ( $0.7596 \pm 0.2919$ ). Medium level was found in heart ( $0.4323 \pm 0.5197$ ), whereas little or no in the remaining tissues. In female, it was high in breast muscle ( $0.3143 \pm 0.1916$ ), heart ( $0.2207 \pm 0.4687$ ), and leg muscle ( $0.2112 \pm 0.6714$ ). Little or no *ATGL* mRNA was found in the remaining tissues (Fig. 6 and Tab. 7).

In this study we cloned the *ATGL* gene cDNA of Chinese francolin, pigeon, bengalese finch and house sparrow. Although we tried many times to clone the complete *ATGL* cDNA

over 88.4%.

The *cfATGL*, *pATGL*, *bfATGL* and *hsATGL* genes expressed mainly in adipose (subcutaneous fat and abdominal fat) and muscle (breast muscle, leg muscle and heart) tissues like in other avian species. The *ATGL* gene of chicken, turkey, quail, parrot and duck all predominantly expressed in adipose tissue – subcutaneous fat, abdominal fat and muscle tissue [Saarela *et al.* 2008, Lee *et al.* 2009, Nie *et al.* 2009, 2010]. Moreover, the *ATGL* gene expression in mammals (humans, mice, pigs and cattle) seems similar to that in avian species, which was also predominantly expressed in adipose, subsequently in muscle tissues [Zimmermann *et al.* 2004, Raben *et al.* 2005, Deuliis *et al.* 2008, 2010, Dai *et al.* 2010]. The fact that *ATGL* was found to be expressed at higher levels in adipose and muscle tissues seems to indicate that the gene was related to fat deposition and carcass traits [Shan *et al.* 2008, Deuliis *et al.* 2010, Dai *et al.* 2010].

In conclusion, the *ATGL* genes of Chinese francolin, pigeon, bengalese-finch and house sparrow identified by this study provide basic information for further characterizing this gene in birds.

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