

cDNA sequence and tissues expression analysis of lipoprotein lipase from common carp (*Cyprinus carpio* Var. Jian)

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Abstract A full-length cDNA coding lipoprotein lipase (LPL) was cloned from liver of adult common carp (*Cyprinus carpio* Var. Jian) by RT-PCR and rapid amplification of cDNA ends (RACE) approaches. The cDNA obtained was 2,411 bp long with a 1,524 bp open reading frame (ORF) encoding 507 amino acids. This amino acid sequence contains two structural regions: N-terminus (24–354 residues) and C-terminus (355–507 residues). Before N-terminus, 1–23 residues is signal peptide, 6–23 residues is transmembrane helix. At N-terminus, some conserved functional sites were found, including two N-linked glycosylation sites Asn⁴¹ and Asn⁸⁸; one catalytic triad Ser¹⁷⁴, Asp¹⁹⁸ and His²⁸³; one conserved heparin-binding site Arg³²¹ to Arg³²⁴ (RKNR); eight cysteines residues Cys⁶⁹ and Cys⁸², Cys²⁵⁸ and Cys²⁸¹, Cys³⁰⁶ and Cys³²⁵, Cys³¹⁷ and Cys³²⁰ which are involved in four disulfide bridges; one polypeptide “lid” that participates in substrate specificity. At C-terminus, Asn⁴⁰¹ is another N-linked glycosylation site, and Trp⁴³⁴ and Trp⁴³⁵ (WW) is lipid-binding site. The amino acid sequence has a high similarity, and shows similar structural features to LPL of other species. Tissue distribution of *LPL* mRNA in liver, head kidney, mesenteric adipose tissue, heart and white muscle of common carp was analyzed by semi-quantitative RT-PCR method using β -actin gene as internal control. The result showed that the expressions of *LPL* mRNA were detected in all examined tissues of common carp. The expression levels of *LPL* in the mesenteric adipose tissue was highest

among these tissues, following in liver and head kidney, and the lowest expression was found in heart and white muscle.

Keywords *Cyprinus carpio* var. Jian · Full-length cDNA · Lipoprotein lipase gene · RACE · Tissues expression

Introduction

Lipoprotein lipase (LPL, EC 3.1.1.34) is a member of the lipase gene superfamily, which initially consisted of three mammalian lipase (pancreatic lipase, PL; lipoprotein lipase, and hepatic lipase, HL) based on amino acid sequence similarity and gene organization [1]. Family size increased when several proteins were subsequently added based on amino acid homology, including PL-related proteins 1 and 2, phosphatidylserine phospholipase A1, and endothelial lipase (EL). All these enzymes would have evolved from a common ancestor and they play a critical role in the absorption and metabolism of lipids and lipoproteins. Lipases are water-soluble enzymes that hydrolyze ester bonds of water-insoluble substrates such as triglycerides, phospholipids, and cholesteryl esters [2, 3].

Mammalian mature LPL is a glycoprotein that is primarily synthesized in adipocytes, muscle cells, macrophages, and lactating mammary gland, but not in the adult liver [4, 5]. In human, LPL gene encoded 475 amino acids, including a 27 amino acids signal peptide. The mature LPL protein consists of 448 amino acids after resecting the signal peptide, which molecular weight is 55 kDa [3]. The enzyme is active as a non-covalent homodimer and is bound to the surface of endothelial cells via glycosaminoglycans, from which it can be released by heparin [6].

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LPL plays a key role in lipid metabolism by hydrolyzing triglycerides in chylomicrons and very low-density lipoproteins (VLDL). It thereby generates non-esterified fatty acids and 2-monoacylglycerol, which are utilized either for storage or energy production [7]. The abnormalities in LPL function have been found to be associated with a number of pathophysiological conditions, including atherosclerosis, chylomicronaemia, obesity, Alzheimer's disease, and dyslipidaemia associated with diabetes, insulin resistance, and infection [6].

LPL full-length cDNA has been cloned and characterized in a number of fish, including zebrafish (*Danio rerio*) [8], rainbow trout (*Oncorhynchus mykiss*) [9, 10], gilthead sea bream (*Sparus aurata*) [1], red sea bream (*Pagrus major*) [11–13], and European sea bass (*Dicentrarchus labrax*) [14]. Furthermore, LPL expression has been measured in a variety of tissues of rainbow trout [9], red sea bream [12, 15, 16] and gilthead sea bream [1]. In contrast to mammals, LPL activity and expression have also been detected in the liver of adult fish [1, 9, 15, 16]. The effects of feeding condition and nutritional state on *LPL* gene expression in the liver, visceral adipose tissue and muscle were investigated. The regulatory effect of dietary lipid level on *LPL* gene expression was tissue-specific and related to feeding conditions. In red sea bream, when fed high-fat diets tended increase the *LPL* mRNA level in the liver, but had no effect in the visceral mesenteric adipose tissues. Starvation (at 48 h post-feeding) drastically stimulated *LPL* gene expression in the liver, but decreased it in visceral mesenteric adipose tissues [13, 15, 17, 18]. In gilthead sea bream, when fed plant-protein-based diets, hepatic *LPL* expression was up-regulated whereas an opposite trend was found in the mesenteric adipose tissue [1]. Fasting for 2 weeks provoked a clear decrease in adipose tissue LPL activity and *LPL* mRNA levels, while no effects were observed in red muscle [19].

Common carp (*Cyprinus carpio* var. Jian) is one of the most popular cultured fishes in China. Some diseases associated with lipid metabolism have been found to increase year after year in artificial culture. The aim of the present work was to clone the full-length cDNA of *LPL* gene from liver of adult common carp and analyze its tissue distribution to explore its role in lipid metabolism. The results will facilitate further studies for the structure, function and regulation of the gene expression of *LPL* in omnivorous fish.

Materials and methods

Fish

Adult common carp (*C. carpio* var. Jian, Cyprinidae, Cypriniformes, Teleostei), which body weight ranged from

750 g to 1,000 g, were collected from a pond in Lian-yungang, Jiangsu province in May, 2007, and transported to the indoor tanks in the Huaihai Institute of Technology, Jiangsu province, China. The fish were maintained in a 1000L tanks under natural conditions of photoperiod fed commercial diet for 2 weeks (May–June). The water temperature ranged from 23 to 27°C.

Total RNA extraction from liver

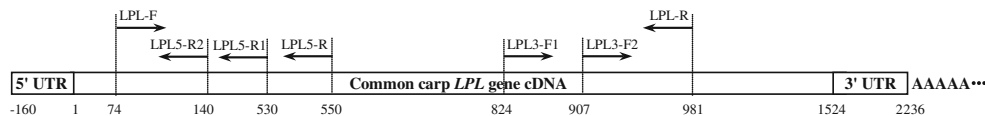
The fish were killed by a blow to the head and liver were rapidly excised and ground sample under liquid nitrogen to a fine power using a mortar. Total RNA was extracted from approximately 50 mg of liver using EZ-10 Spin Column RNA Purification Kit (BBI), according to instructions from the manufacturer. The total RNA was treated using an on-membrane DNase I digestion Kit (OMEGA). The integrity of isolated RNA was assessed by 1% w/v formaldehyde denaturing agarose gels electrophoresis, its quantity and purity was determined by absorbance measures at 260 nm and 280 nm with an UV-Visible spectrophotometer GeneQuant pro (GE), and diluted to 0.5 µg/µl with DEPC-water, then stored at –80°C for future use.

Synthesis of the first-strand cDNA and isolation of core cDNA fragments of *LPL*

Reverse transcription was performed with total RNA from the liver of adult common carp as template and oligo(dT)₁₈ as primer using an AMV First Strand cDNA Synthesis Kit (BBI), according to the manufacturer's instructions. 20 µl reaction volume mixture composed of 5 µl total RNA (0.5 µg/µl), 1 µl Oligo(dT)₁₈ (0.5 µg/µl), 5 µl RNase-free ddH₂O, 4 µl 5× Reaction Buffer, 1 µl RNase inhibitor (20 U/µl), 2 µl dNTP Mix (10 mM each), 2 µl AMV Reverse transcriptase (10 U/µl). The resulting first strand cDNA then be diluted to 1:10 with ddH₂O, and used as a template for PCR. A 909 bp core cDNA fragment of *LPL* was amplified using LPL-F and LPL-R as specific primers (Table 1). Approximate locations of primers were shown in Fig. 1. A pair of primer was designed based on sequence information of zebrafish *LPL* cDNA obtained from GenBank (BC064296, NM_131127), using Primer Premier 5.0 software. PCR was set up in a 50 µl volume reaction mixture composed of 2 µl diluted first strand cDNA product, 5.0 µl 10× buffer (with 20 mM Mg²⁺), 1.0 µl dNTP (10 mM each), 1.0 µl each primer (25 µM), 0.4 µl AmpliTag[®] DNA polymerase (5U/µl BBI), and ddH₂O to 50 µl. Amplification was carried out in a BIO-RAD thermal cycler (iCycler), using the reaction settings as follows: initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturing for 40 s at 94°C, annealing for 40 s at 52°C, extension for 60 s at 72°C, and a final extension for

Table 1 The primers used for *LPL* gene cDNA RT-PCR and RACE

Primers name	Sequence 5' → 3'	Use	Expected product (bp)
LPL-F	CGATTGARCCARCGAGTG	Used with LPL-R for RT-PCR of core fragment	909
LPL-R	CTTGTTGCARCGRTTCTT	Used with LPL-F	
Oligo(dT) ₁₆ AP	CTGATCTAGAGGTACCGGATCC(T) ₁₆	Synthesis of the first-strand cDNA for 3'RACE	
LPL3-F1	TGGATCAGATCGTGAAGTGC	Used with AP for first PCR of 3'RACE	
AP	CTGATCTAGAGGTACCGGATCC	Used with LPL3-F1 or LPL5-R2	
LPL3-F2	GCTTACCCTGCAGCTCCA	Used with Race3-R for nested PCR of 3'RACE	830
Race3-R	CTAGAGGTACCGGATCCTT	Used with LPL3-F2	
LPL5-R	CAGCGATTCTGCTACAT	Synthesis of the first-strand cDNA for 5'RACE	
LPL5-R1	GCACCAAGACTGTAACCC	Used with Oligo(dT) ₁₆ AP for first PCR of 5'RACE	
LPL5-R2	ATCATCCATTCTGTGSCAT	Used with AP for nested PCR of 5'RACE	335
LPL-F1	CATCTGTTGGGTTACAGT	Used with LPL-R for RT-PCR of tissue distribution	478
Actin-F	CGTGATGGACTCTGGTGA	Used with Actin-R as internal standard	438
Actin-R	ACAGTGTGGCATAACAGGT	Used with Actin-F	

**Fig. 1** Primers and approximate locations in *LPL* gene cDNA used for RT-PCR and RACE

7 min at 72°C. Five PCR products were selected by 1% w/v agarose gels electrophoresis and delivered to Shanghai Sangon Biological Engineering and Technology and Service Co. Ltd (Shanghai, China) for sequencing. The sequence was conducted in both forward and reverse directions using the amplification primers by ABI prismTM 377 automatic DNA sequencer. The forward and reverse sequences were assembled using SeqMan II software in DNASTar Package version 5.01, and the core fragment of *LPL* was obtained. According to the sequence information of this fragment, gene-specific primers were designed for 3' RACE and 5' RACE.

Rapid amplification of the 3' end (3' RACE)

Rapid amplification of the 3' end was performed using 3'-Full RACE Core Set (TaKaRa), according to the manufacturer's instructions. The primers used for 3' RACE are shown in Table 1. Firstly, reverse transcription of 2 µg of common carp total RNA was performed with Oligo(dT)₁₆AP as primer, which containing an anchor sequence. The resulting first strand cDNA then be diluted to 1:10 with ddH₂O and used as a template for PCR. Amplification was done with a gene-specific forward primer LPL3-F1 and a reverse primer AP that corresponds to the anchor sequence. After the first PCR, the product was diluted to 1:10 with ddH₂O and used as template for the nested PCR, which was done with a specific forward primer LPL3-F2 and a reverse primer RACE3-R. The first and nested PCR were carried out

under the same condition as above. The nested PCR product was separated by agarose gel electrophoresis and bands of the expected size was excised, and purified using the EZ-10 Spin Column DNA Gel Extraction Kit (BBI). The purified PCR product was subcloned into a PUCm-T vector (BBI) and five independent clones were sequenced in both forward and reverse directions using the universal M13 primers.

Rapid amplification of the 5' end (5' RACE)

Rapid amplification of the 5' end was made following a protocol described by Dieffenbach [20], the primers used for 5' RACE are shown in Table 1. Briefly, reverse transcription of 2 µg total RNA was performed with a specific reverse primer LPL5-R to obtain the first strand cDNA. After RNase H (MBI) treatment, an oligo(dA) tail at the 5' end was added using terminal deoxynucleotidyl transferase (MBI). The resulting product then be diluted to 1:10 with TE buffer, and used as a template for the first PCR. Amplification was done with a universal forward primer Oligo(dT)₁₆AP containing a poly-dT and an anchor sequence and a specific reverse primer LPL5-R1. The first PCR product was diluted to 1:10 with ddH₂O, and used as a template for the nested PCR, which was done with a anchor forward primer AP and a specific reverse primer LPL5-R2. The first and nested PCR were carried out under the same condition as above. The nested PCR product was eluted from 1.5% w/v agarose gel. The purified PCR product was subcloned into a PUCm-T vector (BBI) and five independent clones were sequenced in

both forward and reverse directions using the universal M13 primers.

Sequence analysis

The core fragment, 3' end and 5' end sequences were assembled using SeqMan II software in DNASTar Package to obtain full-length cDNA of *LPL*. The sequence was edited and analyzed using the program EditSeq of DNASTar package to search open reading frame (ORF) and then translate it into amino acid sequence using standard genetic codes. The amino acid sequence of LPL was tested for the presence of signal peptide with SignalP v3.0 at <http://www.cbs.dtu.dk/services/signalP/> [21]. Putative transmembrane regions were predicted with HMMTOP v2.0 at <http://www.enzim.hu/hmmtop/> [22]. The second structure of LPL protein was predicted at <http://www.predictprotein.org/> and <http://www.EMBL-Heidelberg.de/>. The protein sequence alignment was made by program MegAlign of DNASTar package using the Clustal W method. The Phylogenetic tree was produced by Neighbor-Joining (NJ) methods (Kimura 2-parameter model, 10,000 replicates, bootstrap phylogeny test) based on lipoprotein lipase amino acid sequences using MEGA software Version 3.1 [23].

Tissue expression of *LPL* mRNA

Tissue expression of the common carp *LPL* mRNA was determined by semi-quantitative RT-PCR using β -actin as internal control. Total RNAs were extracted from liver, head kidney, mesenteric adipose tissue, heart and white muscle of common carp as mentioned above. The integrity of isolated RNAs were assessed by 1% w/v denaturing agarose gels electrophoresis, whose quantity and purity were determined by absorbance measures at 260 nm and 280 nm, and diluted to 0.5 μ g/ μ l with DEPC-water. Reverse transcriptions were performed with equal quantities of each total RNA (2 μ g) as template and oligo(dT)₁₈ as primer using AMV Reverse Transcriptase. The resulting first strand cDNA from each tissue then be diluted to 1:10 with ddH₂O and used as template for PCRs. The PCRs were carried out using primers LPL-F1 and LPL-R (Table 1) for *LPL* cDNA amplification, Actin-F and Actin-R (Table 1), which were designed based on carp β -actin sequence information (M24113) and spanned a 93 bp intron in genomic DNA, for β -actin cDNA amplification as internal control in different tubes. The PCR products were analyzed by electrophoresis on 1.5% w/v agarose gels and the intensities of PCR bands were quantified using the Quantity one 4.6 system (BIO-RAD, USA). To avoid samples DNA contamination, the negative RT-PCRs control reactions were performed with every total RNA as templates.

Results

Molecular characterization of *LPL*

The full-length cDNA coding lipoprotein lipase (LPL) was completed from liver of adult common carp by assembling the core fragment, 3' and 5' end sequences, and submitted to GenBank (accession numbers **FJ716101**). The complete nucleotide sequence covered 2411 bp with an open reading frame (ORF) of 1,524 bp encoding 507 amino acids residues, including a putative signal peptide of 23 amino acids in length (Fig. 2). The 5'-untranslated region (UTR) covered 160 nt. An ATG initiation codon was found 160 nt downstream of the 5'-start, and a TAA stop codon was present 727 nt upstream of the 3'-end. The 3'-untranslated region (UTR) covered 727 nt containing one AACAAA motifs, which represent putative polyadenylation signals (nt 2,303–2,308).

The LPL protein has a calculated molecular weight of 57.8-kDa and isoelectric point (PI) of 8.531. This amino acid sequence contains two structural regions: N-terminus (24–354 residues) and C-terminus (355–507 residues). Before N-terminus, 1–23 residues is signal peptide, 6–23 residues is transmembrane helix. At N-terminus, some conserved functional sites were found, including two N-linked glycosylation sites Asn⁴¹ and Asn⁸⁸; one catalytic triad Ser¹⁷⁴, Asp¹⁹⁸ and His²⁸³; one conserved heparin-binding site Arg³²¹ to Arg³²⁴ (RKNR); eight cysteines residues Cys⁶⁹ and Cys⁸², Cys²⁵⁸ and Cys²⁸¹, Cys³⁰⁶ and Cys³²⁵, Cys³¹⁷ and Cys³²⁰ which are involved in four disulfide bridges; one polypeptide “lid” that participates in substrate specificity. At C-terminus, Asn⁴⁰¹ is another N-linked glycosylation site, and Trp⁴³⁴ and Trp⁴³⁵ (WW) is lipid-binding site. The amino acid sequence has a high similarity, and shows similar structural features to LPL of other species. Complete amino acid sequence alignment of LPL showed the best identity with zebrafish (the homology is 88.0%), while less identity with human (the homology is 58.3%).

Phylogenetic analysis based on LPL amino acid sequences

Phylogenetic analysis among six fish species and thirteen endotherms species based on lipoprotein lipase amino acids sequences was shown in Fig. 3. Tree topology showed that all fish species were in the same group forming a clade, which were distinguished from endotherms. In fish, red sea bream (*Pagrus major*) was first grouped with gilthead sea bream (*Sparus aurata*), which belong to the same family Sparidae with a high bootstrap value of 99%, and then formed a clade with European seabass (*Dicentrarchus labrax*), which belong to the same suborder Percoidei with

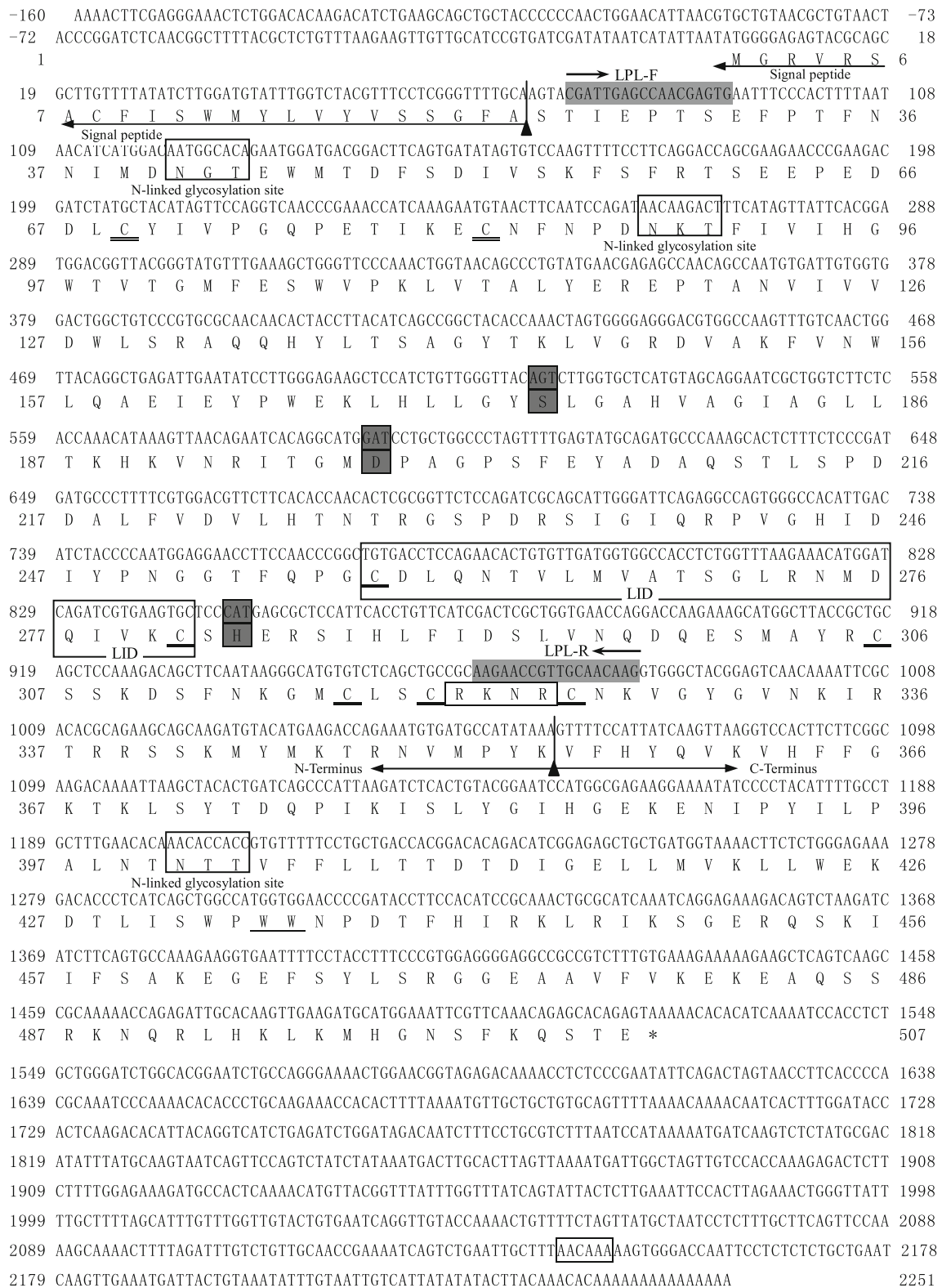


Fig. 2 The nucleotide sequence of *LPL* gene full-length cDNA and the deduced amino acid sequence of *Cyprinus carpio* var. Jian. The grey bases indicate the primers used for RT-PCR. An asterisk represents a termination codon. The boxed amino acid sequences [RKNR] indicate the heparin-binding site. Catalytic triad is boxed in black squares. Four pairs of cysteine residues sites are double

underlined. The underlined amino acid sequences WW indicate the lipid-binding site. Other functional sites, such as N-linked glycosylation sites, signal peptide, polypeptide lid, the boundary of N-terminus and C-terminus structural regions were showed on figure. The nucleotide sequence was submitted to the NCBI GenBank, accession no. **FJ716101**

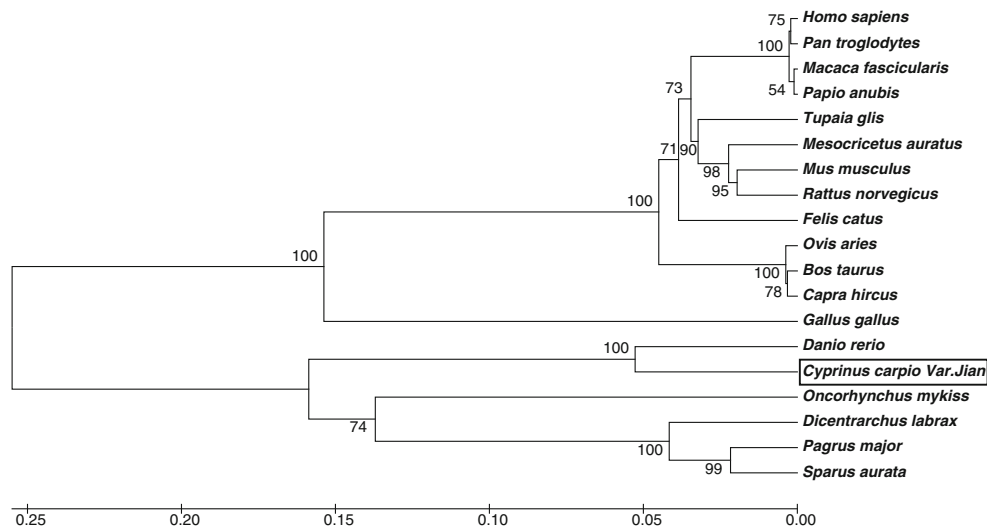


Fig. 3 Phylogenetic tree based on lipoprotein lipase amino acid sequences made with MEGA 3.1 software using Neighbor-Joining (NJ) method. The distance matrix was calculated using the Kimura's 2-parameter model. The numbers represent bootstrap percentages. The topology was tested using bootstrap analyses (10,000 replicates). GenBank accession numbers: *Homo sapiens* **BAD96184**, *Pan troglodytes* **XP_001149804**, *Macaca fascicularis* **BAE90068**, *Papio anubis* **NP_001106082**, *Tupaia glis* **ABQ24000**, *Mesocricetus*

auratus **BAD69621**, *Mus musculus* **BAE29770**, *Rattus norvegicus* **NP_036730**, *Felis catus* **NP_001036032**, *Ovis aries* **NP_001009394**, *Bos taurus* **NP_001068588**, *Capra hircus* **ABD04039**, *Gallus gallus* **NP_990613**, *Cyprinus carpio* var. Jian **FJ716101** which is boxed to show present study, *Danio rerio* **NP_571202**, *Oncorhynchus mykiss* **NP_001118076**, *Dicentrarchus labrax* **CAL69901**, *Pagrus major* **BAE95413**, *Sparus aurata* **AAS75120**

a bootstrap value of 100%. Common carp (*Cyprinus carpio* Var. Jian) and zebrafish (*Danio rerio*), which belong to the same family Cyprinidae, formed another clade with a high bootstrap 100%. In endotherms, human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), crab-eating macaque (*Macaca fascicularis*) and olive baboon (*Papio anubis*), which belong to primates, formed a clade with a bootstrap 100%. Whereas, sheep (*Ovis aries*), cattle (*Bos taurus*) and goat (*Capra hircus*), which belong to ruminants, formed another clade with a bootstrap 100%. Then all species of mammals were in the same group forming a clade, which were distinguished from chicken (*Gallus gallus*). The phylogenetic relationship based LPL amino acid sequences was agreed to traditional classification.

Tissue expression of the common carp *LPL* mRNA

Tissue expression of *LPL* mRNA in liver, head kidney, mesenteric adipose tissue, heart and white muscle of adult common carp was determined by semi-quantitative RT-PCR using β -actin as internal control. The results showed that the amplification yielded 478 bp bands for *LPL* and 438 bp bands for β -actin. RT-PCR analysis revealed that the expressions of *LPL* gene were detected in all examined tissues of adult common carp (Fig. 4a), while the negative control exhibited no products (data not shown). The expression levels of *LPL* in the mesenteric adipose tissue were highest among these tissues, following

in liver and head kidney, and the lowest expression was found in heart and white muscle. The levels of *LPL* mRNA in mesenteric adipose tissue were 1.28, 1.25, 2.79 and 2.92 fold higher than in liver, head kidney, heart and white muscle, respectively. (Fig. 4b).

Discussion

Sequence comparisons to *LPL* from endotherms

In the present study, we cloned and characterized the full-length cDNA coding *LPL* from liver of adult common carp, and analyzed its distribution patterns to investigate its possible roles in lipid metabolism. The cDNA encoded 507 amino acids residues, including a putative signal peptide of 23 amino acids in length, which sequence is very different from other species. The entire mature protein (484 amino acids in length) has a high similarity, and shows similar structural features to *LPL* of other species. The amino acid sequence of common carp *LPL* was compared with the corresponding sequences from human, chicken, zebrafish, rainbow trout, European sea-bass, gilthead sea bream, and red sea bream. The result was shown in Fig. 5.

LPL is a noncovalent dimer of two identical subunits that are held together in a head-to-tail arrangement [3]. In human, three amino acid residues Ala²⁰³, Gly²¹⁵ and Gly²²²

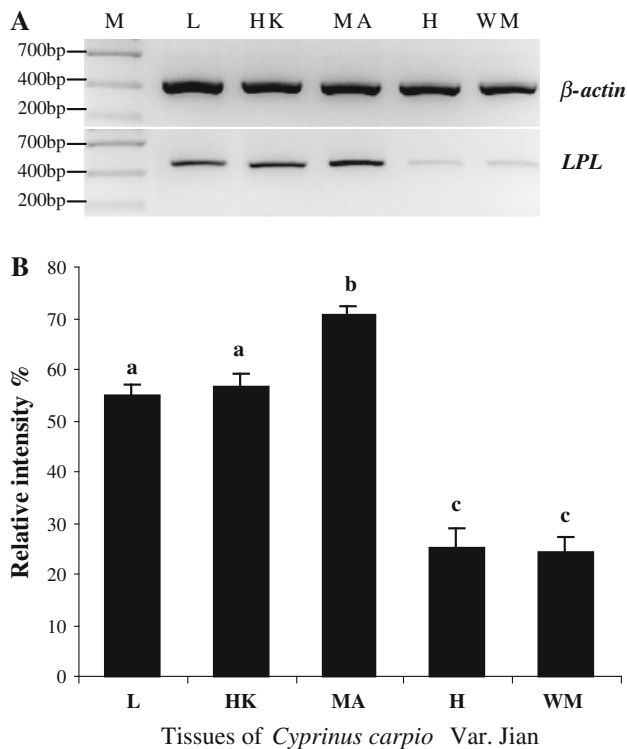


Fig. 4 Expression analysis of *LPL* mRNA in different tissues of *Cyprinus carpio* performed by semi-quantitative RT-PCR with β -actin as internal control. **a** RT-PCR (30 cycles) products analyzed by agarose gel electrophoresis. **b** Relative expression profile of *LPL* normalized with β -actin level. Data points represent the mean values \pm SE of the three replicates with different letters indicate significant different ($P < 0.05$). M, marker; L, liver; HK, head kidney; MA, mesenteric adipose tissue; H, heart; WM, white muscle

have been proven to be critical to produce dimer, and to be found very conserved among endothermy species [5]. These amino acid residues are also found in common carp LPL (Ala²¹⁸, Gly²³⁰, Gly²³⁷). The LPL subunit contain several functional sites: the catalytic triad (Ser, Asp, His), the lipid binding site(s), the heparin binding site(s), and the site for interaction with the activator apolipoprotein CII (apoCII). In human, the N-terminal region contains the conserved serine, aspartic acid and histidine catalytic triad (Ser¹⁵⁹, Asp¹⁸³ and His²⁶⁸, human numbering), where the triacylglycerol hydrolysis is carried out [5]. All of these residues are conserved in all species including common carp LPL. In human, tryptophan clusters at Trp⁴¹⁷, Trp⁴²⁰ and Trp⁴²¹ (human numbering) play an important role in binding to lipid substrates and lipoprotein. The Lys⁴³⁴ as a site for interaction with low-density lipoprotein receptor-related protein (LRP) [5]. All of these residues are conserved in all species including common carp. In human, the putative heparin binding located some positively charged basic residues rich regions, including from Arg³⁰⁶–Arg³⁰⁹, Arg³²¹–Lys³²⁷, and the discontinuous sequence of Lys³⁴⁶

and Lys⁴³⁰–Lys⁴⁴¹ [5]. The corresponding regions in the common carp LPL also contained the positively charged basic residues except for one (Lys⁴⁴⁰, human numbering). Especially, the region Arg³⁰⁶ to Arg³⁰⁹ (RKNR), and positively charged basic residues Arg³²¹, Arg³²⁴, Lys³²⁷, Lys³⁴⁶, Arg⁴³², and Lys⁴³⁴ (human numbering) are very conserved in all species including common carp. LPL requires a specific cofactor, apoC-II, to hydrolyze triglycerides in chylomicrons. In human, the study of chimeric lipases suggested that two regions, Val⁹²–Leu⁹⁵ and Tyr¹⁰⁰–Ser¹⁰⁶ in the N-terminal domain of LPL, were responsible for cofactor activation [24, 25]. The amino acid residues Val⁹²–Leu⁹⁵ (VPKL, human numbering) is conserved in all species including common carp, but the residues Tyr¹⁰⁰–Ser¹⁰⁶ (human numbering) is very different between endotherms and fish species.

LPL is an N-linked glycoprotein, and the consensus sequence for an N-glycosylation site is given as N-X-S/T (X is not P). There are two N-glycosylation sites, Asn⁷⁰ and Asn³⁸⁶ in human LPL [5]. However, the numbers and positions of potential N-linked glycosylation sites are different among species. Three potential N-glycosylation sites are contained in the common carp LPL, in which, the first site Asn⁴¹ (common carp numbering) is conserved among teleost fish which is not found in human and chicken. The second site Asn⁸⁸ (common carp numbering) was found only in common carp. The third site Asn⁴⁰¹ (common carp numbering) in C-terminus region is completely conserved among all species including human and chicken. Whereas, one N-linked glycosylation site Asn⁴⁸⁰ (rainbow trout numbering) was found only in carnivorous fish. We postulate that this site may be related to the carnivorous of fish, but need to be verified.

Human LPL contain ten cysteines residues (Cys⁵⁴ and Cys⁶⁷; Cys²⁴³ and Cys²⁶⁶; Cys²⁹¹ and Cys³¹⁰; Cys³⁰² and Cys³⁰⁵; Cys⁴⁴⁵ and Cys⁴⁶⁵, human numbering) involving in five disulfide bridges. One of the disulfide bridges is located in the C-terminal folding region (Cys⁴⁴⁵ and Cys⁴⁶⁵) [5]. Common carp LPL contains eight cysteines residues in the N-terminal region, but excludes any cysteines in the C-terminal region. This case was found in other fish, such as rainbow trout and red sea bream [9, 13]. This pair of residues in the C-terminal region are conserved in LPL from all mammals, and has been shown to be important for the interaction of LPL with emulsified lipids and also for the stability at 37°C of the LPL dimer [26]. The stability of fish LPL at 37°C was markedly low in comparison with bovine and human LPL. A possible reason for the difference might be that fish LPL does not have the C-terminal disulfide. Fish are poikilothermal vertebrates resulting in a body temperature lower than 37°C. This may have been a necessary adaptation for function at higher body temperatures [9].

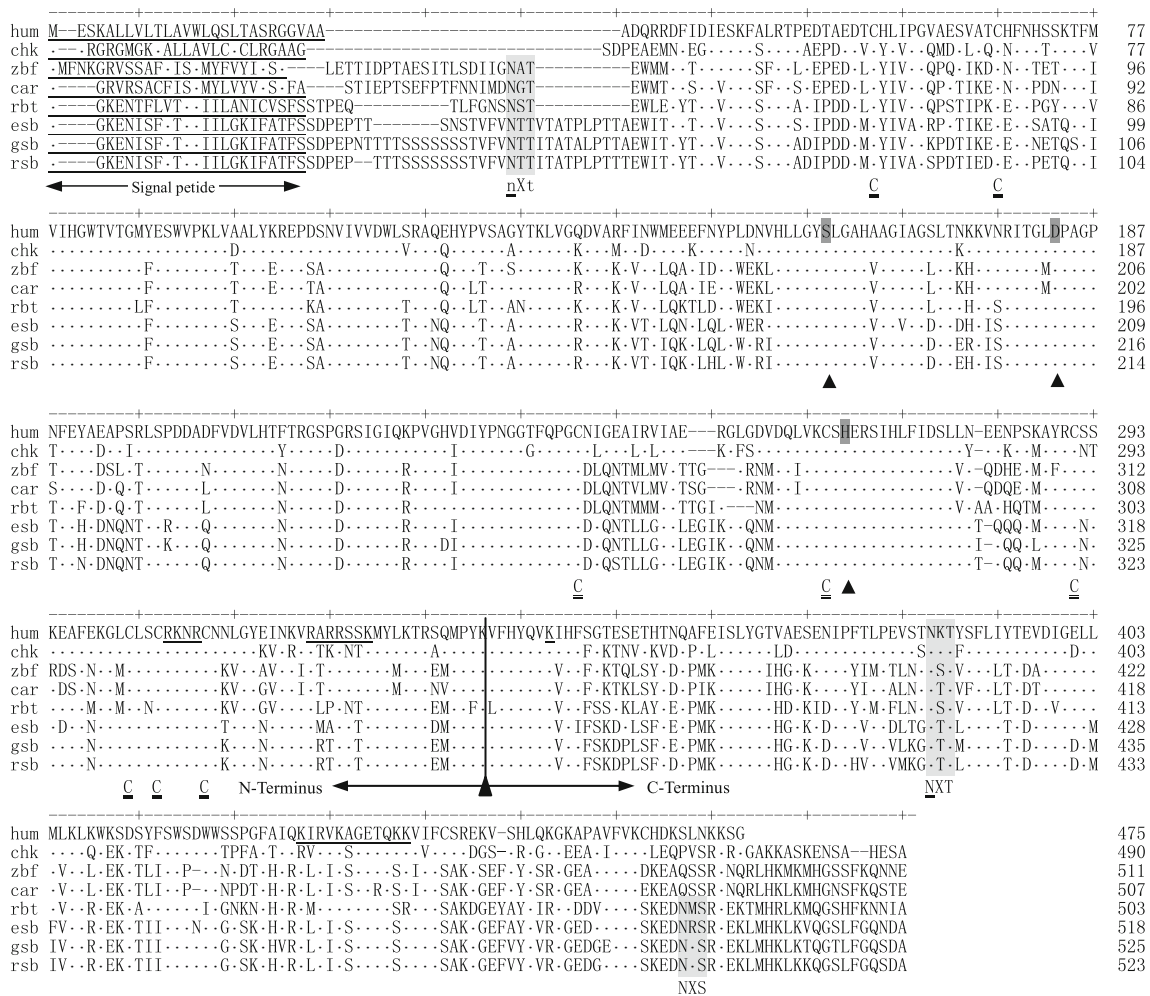


Fig. 5 Multiple alignment of LPL amino acid sequence. Common carp (car, **FJ716101**), human (hum, **BAD96184**), chicken (chk, **NP_990613**), zebrafish (zbf, **NP_571202**), rainbow trout (rbt, **NP_001118076**), European seabass (esb, **CAL69901**), gilthead sea bream (gsb, **AAS75120**), and red sea bream (rsb, **BAE95413**). Sites with an amino acid identical to that on the *top line* (human) indicated with a *dot*. Alignment gaps indicated by *dashes*. Amino acid

substitutions indicated by the respective amino acid. The signal peptide, conserved cysteine residues (C), active site residues (▲), putative heparin binding domains (underline) and a potential conserved N-linked glycosylation site in all sequences (N), a N-linked glycosylation site in all fish (n), and a N-linked glycosylation site only found in carnivorous fish (N) are indicated on figure

Tissue expression of *LPL* mRNA

In the present study, tissue expression of *LPL* mRNA in liver, head kidney, mesenteric adipose tissue, heart and white muscle of adult common carp, was determined by semi-quantitative RT-PCR approach. The result showed that this gene is ubiquitously expressed in all tissues tested, even in liver (Fig. 4a). In mammals, Hepatic *LPL* expression is terminated soon after birth [27]. But in contrast to what has been found in mammals, adult fish, such as rainbow trout, gilthead sea bream, and red sea bream, not only visceral adipose tissue but also liver showed substantial *LPL* gene expression [1, 9, 13, 16]. This indicates *LPL* may have a function in fish liver that in higher vertebrates may be fulfilled by the related hepatic lipase. Our

present data showed that the expression of *LPL* is tissue-specific, in which the highest expression was found in mesenteric adipose tissue, following in head kidney and liver, and the lowest expression was found in heart and white muscle. A graded tissue expression of *LPL* (adipose tissue > liver > white skeletal muscle) was also found in rainbow trout and gilthead sea bream [1, 9]. In gilthead sea bream, the expression of *LPL* in mesenteric adipose tissue was several times higher than in liver and skeletal muscle.

In summary, in this study we have cloned and characterized the cDNA encoding *LPL* from the liver of the omnivorous common carp. The main domains (catalytic site, disulfide bridge, N-linked glycosylation site, heparin binding domain, lipid binding site, and site of dimer formation) are basically conserved between the common carp

and other vertebrates. Our results demonstrate that the liver is one of the main LPL producing tissue in adult fish.

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References

- Saera-Vila A, Calduch-Giner JA, Gomez-Requeni P et al (2005) Molecular characterization of gilthead sea bream (*Sparus aurata*) lipoprotein lipase. Transcriptional regulation by season and nutritional condition in skeletal muscle and fat storage tissues. *Comp Biochem Physiol B Biochem Mol Biol* 142:224–232
- Mukherjee M (2003) Human digestive and metabolic lipases—a brief review. *J Mol Catal B* 22:369–376
- Wong H, Schotz MC (2002) The lipase gene family. *J Lipid Res* 43:993–999
- Wion KL, Kirchgessner TG, Lusis AJ et al (1987) Human lipoprotein lipase complementary DNA sequence. *Science* 235:1638–1641
- Raisonnier A, Etienne J, Arnault F et al (1995) Comparison of the cDNA and amino acid sequences of lipoprotein lipase in eight species. *Comp Biochem Physiol B Biochem Mol Biol* 111:385–398
- Mead JR, Irvine SA, Ramji DP (2002) Lipoprotein lipase: structure, function, regulation, and role in disease. *J Mol Med* 80:753–769
- Wang CS, Hartsuck J, McConathy WJ (1992) Structure and functional properties of lipoprotein lipase. *Biochimica et Biophysica Acta* 1123:1–17
- Arnault F, Etienne J, Noe L et al (1996) Human lipoprotein lipase last exon is not translated, in contrast to lower vertebrates. *J Mol Evol* 43:109–115
- Lindberg A, Olivecrona G (2002) Lipoprotein lipase from rainbow trout differs in several respects from the enzyme in mammals. *Gene* 292:213–223
- Kwon JY, Prat F, Randall C et al (2001) Molecular characterization of putative yolk processing enzymes and their expression during oogenesis and embryogenesis in rainbow trout (*Oncorhynchus mykiss*). *Biol Reprod* 65:1701–1709
- Oku H, Ogata HY, Liang XF (2002) Organization of the lipoprotein lipase gene of red sea bream *Pagrus major*. *Comp Biochem Physiol B Biochem Mol Biol* 131:775–785
- Oku H, Koizumi N, Okumura T et al (2006) Molecular characterization of lipoprotein lipase, hepatic lipase and pancreatic lipase genes: effects of fasting and refeeding on their gene expression in red sea bream *Pagrus major*. *Comp Biochem Physiol B Biochem Mol Biol* 145:168–178
- Liang XF, Oku H, Yo H et al (2002) The cDNA sequence and tissue expression of lipoprotein lipase gene of a marine fish, red sea bream (*Pagrus major*). *Chinese Journal of Biochemistry and Molecular Biology* 18:712–719
- Jose Ibanez A, Peinado-Onsurbe J, Sanchez E et al (2008) Lipoprotein lipase (LPL) is highly expressed and active in the ovary of European sea bass (*Dicentrarchus labrax* L.), during gonadal development. *Comp Biochem Physiol A Mol Integr Physiol* 150:347–354
- Liang XF, Ogata HY, Oku H (2002) Effect of dietary fatty acids on lipoprotein lipase gene expression in the liver and visceral adipose tissue of fed and starved red sea bream *Pagrus major*. *Comp Biochem Physiol A Mol Integr Physiol* 132:913–919
- Liang XF, Oku H, Ogata HY (2002) The effects of feeding condition and dietary lipid level on lipoprotein lipase gene expression in liver and visceral adipose tissue of red sea bream *Pagrus major*. *Comp Biochem Physiol A Mol Integr Physiol* 131:335–342
- Liang XF, Bai JJ, Lao HH et al (2003) Nutritional regulation of lipoprotein lipase gene expression and visceral fat deposition in red sea bream (*Pagrus major*). *Oceanologia et limnologia Sinica* 34:625–633
- Liang XF, Li YQ, Li GS et al (2004) Cis-acting element and in vivo regulation of lipoprotein lipase gene of red sea bream *Pagrus major*. *Journal of tropical oceanography* 23:49–55
- Albalat A, Saera-Vila A, Capilla E et al (2007) Insulin regulation of lipoprotein lipase (LPL) activity and expression in gilthead sea bream (*Sparus aurata*). *Comp Biochem Physiol B Biochem Mol Biol* 148:151–159
- Dieffenbach CW, Dveksler GS (1995) PCR primer: a laboratory manual. Cold Spring Harbor Laboratory Press Plainview, N.Y
- Bendtsen JD, Nielsen H, von Heijne G et al (2004) Improved prediction of signal peptides: SignalP 3.0. *J Mol Biol* 340:783–795
- Tusnady GE, Simon I (2001) The HMMTOP transmembrane topology prediction server. *Bioinformatics* 17:849–850
- Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 5:150–163
- Bruin T (1994) Human lipoprotein lipase: molecular genetics and structure function analysis. Thesis Publishers, Amsterdam
- McIlhargey TL, Yang Y, Wong H et al (2003) Identification of a lipoprotein lipase cofactor-binding site by chemical cross-linking and transfer of apolipoprotein C-II-responsive lipolysis from lipoprotein lipase to hepatic lipase. *J Biol Chem* 278:23027–23035
- Keiper T, Schneider JG, Dugi KA (2001) Novel site in lipoprotein lipase (LPL415–438) essential for substrate interaction and dimer stability. *J Lipid Res* 42:1180–1186
- Staels B, Auwerx J (1992) Perturbation of developmental gene expression in rat liver by fibric acid derivatives: lipoprotein lipase and alpha-fetoprotein as models. *Development* 115:1035–1043