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Isolation, Molecular Phylogeny, and Tissue Distribution of Four cDNAs Encoding the Apolipoprotein Multigene Family in Barred Knifejaw, *Oplegnathus fasciatus* (Teleostei, Perciformes)

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Lipoproteins are complexes of lipids and specific apolipoproteins that are involved in lipid transport and redistribution among various tissues. In this study, we isolated full-length apolipoprotein cDNA sequences encoding apolipoprotein A-I (apoA-I), apoE, apoC-II, and apo-14 kDa in barred knifejaw, *Oplegnathus fasciatus*. In addition, we reconstructed phylogenetic trees and investigated mRNA tissue distributions. Alignment analyses of amino acid sequences revealed that secondary structures of the polypeptides apoA-I, apoE, and apoC-II in barred knifejaw are well conserved with their teleostean and mammalian counterparts in terms of characteristic tandem repetitive units forming amphipathic α-helices. Both the sequence alignment data and cleavage sites of apo-14 kDa indicated a clear differentiation between Percomorpha and Cypriniformes. Meanwhile, the phylogenetic trees of apolipoprotein sub-families suggested that the common ancestor prior to the split of the Actinopterygii (ray-finned fishes) and Sarcopterygii (tetrapods) would have possessed the primordial protein-encoding genes. Tissue distribution of each apolipoprotein transcript determined by semi-quantitative RT-PCR showed that barred knifejaw apoA-I transcripts were more or less ubiquitously expressed in the liver, intestines, brain, muscle, spleen, and kidney. The most striking difference from previous observations on barred knifejaw was the ubiquitous expression of apoE across all somatic tissues. Barred knifejaw apoC-II showed tissue-specific expression in the liver and intestines, while the liver and brain were the major sites of apo-14 kDa mRNA synthesis.

Key words: Apolipoproteins, Barred knifejaw, cDNAs, mRNA, Phylogeny, Tissue distribution

Introduction

Lipoproteins are complexes of lipids and specific protein components called apolipoproteins. Apolipoproteins play an important role in lipid transport and redistribution among various tissues, the maintenance of lipoprotein structures, and the modulation of enzymes involved in lipid metabolisms (Mahley et al., 1984; Li et al., 1988). Eight apolipoproteins in mammals [apolipoprotein A-I (apoA-I), apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III, apoC-IV, and apoE] share characteristic structural units of repeated amphipathic α-helical segments. These proteins form a multigene family that has evolved from a common ancestral apolipoprotein gene through whole-gene duplications, intraexonic amplifications of repeating units, and intragenic deletions (Boguski et al., 1985; Rajavashisth et al., 1985; Li et al., 1988).

In contrast to the wealth of information on the structure and function of apolipoproteins in advanced vertebrates, relatively few studies have examined apolipoprotein genes in teleosts, the evolutionary lower and largest vertebrate group. Previous studies have reported isolations of apolipoprotein cDNA sequences in rainbow trout, *Oncorhynchus mykiss* (apoA-I, Delcuve et al., 1992; apoC-II, Shen et al., 2000), Atlantic salmon, *Salmo salar* (apoA-I, Powell et al., 1991), zebrafish, *Danio rerio* (apoA-I and apoE, Babin et al., 1997), gilthead seabream, *Sparus aurata* (apoA-I, Llewellyn et al., 1998), Japanese eel, *Anguilla japonica* (apoA-I, apoA-IV and apo-14 kDa, Kondo et al., 2001), orange-spotted grouper, *Epinephelus coioides* (apo-14 kDa, Zhou et al., 2005),

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and mud loach, *Misgurnus mizolepis* (apoA-I, Lee et al., 2007). As many as nine apolipoprotein genes (four apoA-IV isoforms, two apoE isoforms, and a single isoform each of apoA-I, apoC-II, and apo-14 kDa) have been isolated and characterized in pufferfish, *Takifugu rubripes* (Kondo et al., 2005). Among them, apo-14 kDa was reported to be fish-lineage specific (Kondo et al., 2001, 2005). Recent studies have suggested that certain apolipoproteins may play an important role in the fish innate immune system (Concha et al., 2003; Villarroel et al., 2007) and morphogenic homeostasis during embryonic and ontogenic development (Lang et al., 2005; Zhou et al., 2005). In addition, several apolipoprotein members are sensitively regulated during exposure to xenobiotics and pollutants (Pinto et al, 2006; Wintz et al., 2006; Martyniuk et al., 2007).

Previous reports have claimed that fish apolipoprotein genes have low levels of homology with their mammalian counterparts. Such a low genetic homology between fish and mammals, as well as the discovery of a fish-specific apolipoprotein (i.e., apo-14 kDa) indicates that fish may have experienced a different evolutionary history than mammals with regard to apolipoproteins (Kondo et al., 2005). Fish have acquired different physiological mechanisms for the transport and use of lipid molecules. Marine fishes use lipids as their major energy source, unlike mammals, which mainly use carbohydrates. Thus, lipid metabolism is more important for homeostasis maintenance in poikilotherms than homeotherms (Kondo et al., 2005).

Barred knifejaw, *Oplegnathus fasciatus* (Teleostei, Perciformes), is one of the most highly valued marine fish species, and information regarding the genetic determinants of apolipoproteins would provide a strong basis for understanding lipoprotein physiology in this species. The aim of this study was to isolate and characterize full-length cDNA sequences encoding apoA-I, apoE, apoC-II, and apo-14 kDa in barred knifejaw, and to reconstruct phylogenetic trees inferred from these amino acid sequences. Furthermore, we investigated the tissue distributions of apolipoprotein transcripts using a semi-quantitative RT-PCR assay.

Materials and Methods

Fish and tissue sampling

Barred knifejaw juveniles (average body weight of 180±25 g) were purchased from a local supplier. Various tissues (brain, eyes, fins, gills, heart, intestines, kidney, liver, muscle, and spleen) were surgically removed from 12 individuals and pooled within each tissue type. Total RNA was extracted from each tissue using TriPure Reagent (Roche, Germany) according to the manufacturer's instruction. Total RNA was purified using the RNeasy® Min-Elute™ Cleanup kit (Qiagen, Germany) that included a DNase I treatment as manufacturer's suggestion. RNA integrity was determined based on the 28S:18S rRNA ratio using ethidium bromide (EtBr)-stained 1% formaldehyde-MOPS gels. Quantity and purity of the total RNA samples were checked by spectrophotometry.

Isolation of full-length apolipoprotein cDNA sequences

From our expressed sequence tag (EST) analyses on liver and kidney cDNA libraries in barred knifejaw (unpublished data), EST clones that showed significant homology to previously known vertebrate apolipoprotein members were selected. Of more than 2,000 ESTs, 83 transcripts were matched with four apolipoprotein orthologs based on BLAST searches in GenBank (http://www.ncbi.nlm.nih.gov/blast). Each sequence was imported into a nucleotide sequence analysis software Sequencher (Gene Codes Co., USA) for sequence editing and contig assembly. Open reading frame (ORF) sequence of each contig was reconfirmed by RT-PCR amplification followed by TA-cloning using the pGEM®-T Easy Vector System (Promega, USA) and sequence analysis. The apolipoprotein sequences analyzed in this study are available in GenBank under accession numbers EU812516 to EU812519.

Analysis of amino acid sequence, multiple sequence alignment, and molecular phylogeny

Four barred knifejaw apolipoprotein cDNAs were translated into amino acid (AA) sequences using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf). The most likely cleavage sites for the signal peptide were predicted using the SignalP 3.0 Server (http://www.cbs.dtu.dk/services/SignalP/). The molecular weights and pI values were predicted using the ExPASy Proteomics Server (http://www.expasy.ch/tools/pi_tool.html).

Complete cDNA sequences of apolipoprotein genes for teleosts, and representative bird and mammals were obtained from GenBank. Multiple sequence alignments were carried out in three separate multigene subfamilies using BioEdit 7.0.8 (Hall, 1999). First, AA sequence data for teleost apoA-I and apoE were aligned with their mammalian and avian homologs in accordance with the repeat

units. Secondly, AA sequences of teleost apoC-II were aligned together with the representative mammalian counterparts (apoC-I to apoC-III). Finally, barred knifejaw apo-14 kDa was aligned with teleostean homologs. For each alignment matrix, the signal peptide region, propeptide and N-terminal regions, 33-AA unit, and 11- and 22-AA repeat units were identified and refined according to Kondo et al. (2005). Sequence Identity Matrix in BioEdit was used to calculate similarities among AA sequences.

Matrices of three multigene subfamilies were separately imported into MEGA 4.01 (Tamura et al., 2007) to reconstruct molecular phylogenetic trees. An unrooted neighbor-joining (NJ) tree was reconstructed using the Poisson correction model for AA substitutions. The robustness of the tree topology was estimated by bootstrap analyses with 1,000 pseudoreplicates.

mRNA tissue distribution using a semi-quantitative RT-PCR assay

A semi-quantitative RT-PCR assay was carried out to examine mRNA tissue distribution of each apolipoprotein gene across ten somatic tissues. Based on preliminary experiments that optimized the conditions for RT-PCR amplification, 4 μg of each total RNA were reverse-transcribed at 37°C for 1 hr using an oligo $d(T)_{20}$ primer at 1 μM and Omni-transcriptase (Qiagen, Germany) in the 40-μL reaction volume according to the manufacturer's recommendations. To prepare an internal control, a RB 18S rRNA 1R primer (unpublished data) was also included at 0.1 μM of the final concentration in the RT-reaction. One μL of RT-product (cDNA) was subjected to PCR amplification for each apolipoprotein gene and internal control (18S rRNA). RT-PCR primers, thermal cycling conditions, and the expected size of the PCR product for each apolipoprotein gene are given in Table 1. PCR was carried out using *AccuPower*® HotStart PCR PreMix (Bioneer, Korea), and 5 μL of each PCR-amplified product was separated on a 1.2% agarose gel. EtBr-stained bands were analyzed by image analysis software Quantity-One®, implemented in VersaDoc 4000 (Bio-Rad, USA). Relative mRNA levels of each apolipoprotein gene were determined based on normalization against 18S rRNA bands. Triplicate assays were performed for each tissue. Statistical evaluation was performed using an analysis of variance (ANOVA) followed by Duncan's multiple range tests. The significance level for all tests was set at P=0.05.

Results and Discussion

Characteristics of apolipoprotein cDNA and deduced amino acid sequences

Four cDNA sequences encoding apoA-I, apoC-II, apoE, and apo-14 kDa were isolated and characterized from barred knifejaw cDNA libraries. Information on their full-length cDNA sequences is summarized in Table 2. ApoA-IV and apoA-II, which have been reported in pufferfish (Kondo et al., 2005) and common carp (Concha et al., 2003), respectively, were not isolated from our EST database search. In addition, the presence of multiple isoforms within each apolipoprotein gene as exemplified in pufferfish, rainbow trout, and zebrafish was not found in this study.

All four full-length cDNAs of barred knifejaw apoA-I, apoE, apoC-II, and apo-14 kDa comprised ORFs of 786, 828, 291, and 429 bp, respectively (Table 2), and all possessed the canonical polyadenylation signal aataaa. The mature proteins of

Table 1. Oligonucleotide primers and thermal cycling conditions used for semi-quantitative PCR assays to detect apolipoprotein mRNA transcripts of barred knifejaw, *Oplegnathus fasciatus*, across tissues. [1]Each PCR reaction was carried out with an initial denaturation step at 94°C for 10 min prior to thermal cycling

Gene	Primer name	Sequence (5' to 3')	Amplicon (bp)	Thermal cycling condition[1]
apoA-I	RBapoA-I F RBapoA-I R	ATCGCTTCTACCGTCACCAA ACACAGAGCTTCAGGCACTT	379	26 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 45 s
apoE	RBapoE F RBapoE R	ACCAAGAAGCTGAGCGACAT GGTCTCCTTGACCTTGTCCA	228	26 cycles of 94°C for 20 s, 58°C for 20 s, and 72°C for 20 s
apoC-II	RBapoC-II F RBapoC-II R	GTATCTCACCTCACCCAAGA ACCCATGGAGAAATTGGCTG	252	27 cycles of 94°C for 20 s, 58°C for 20 s, and 72°C for 30 s
apo-14 kDa	RBapo14kD F RBapo14kD R	AGCGAGAGTGGACAGAGAGC TACTCAGCGGGCAGAACTTT	239	26 cycles of 94°C for 20 s, 58°C for 20 s, and 72°C for 30 s
18S rRNA	RB18S F RB18S R	TACCACATCCAAGGAAGGCA TTCCTAGCTGCGGTATTCAG	407	20 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s

Table 2. Sequence characteristics of apolipoprotein cDNA and deduced amino acid sequences of barred knifejaw, *Oplegnathus fasciatus*. [1]Including poly (A+) tail; [2]Excluding the stop codon; [3]Excluding poly (A+) tail

Gene	Total cDNA (bp)[1]	5'-UTR (bp)	ORF (bp)[2]	Deduced amino acids (aa)	3'-UTR (bp)[3]	Polyadenylation signal	Signal peptide (aa)	Predicted molecular weight (kDa)	Theoretical pI value
apoA-I	1,255	23	786	262	422	aataaa	18	29.10	5.29
apoE	1,630	36	828	276	737	aataaa	18	31.06	4.93
apoC-II	877	46	291	97	521	aataaa	20	10.90	5.05
apo-14 kDa	797	53	429	143	287	aataaa	20	15.64	6.14

apoA-I, apoE, apoC-II, and apo-14 kDa have the predicted molecular weights of 29.10, 31.06, 10.90, and 15.64 kDa, respectively, and theoretical pI values of 5.29, 4.93, 5.05, and 6.14, respectively. From extensive homology searches against GenBank, the AA sequences of barred knifejaw apoA-I and apoE showed the highest similarities to those of European flounder, *Platichthys flesus* (60.4%) and rainbow trout (59.2%), respectively. Barred knifejaw apoC-II showed the highest similarity to a perciform species, gilthead seabream (74.2%). An average of 22.3% similarity to mammalian apoC-IIs and 13.1-16.5% similarities to mammalian apoC-Is and apoC-IIIs were also observed. Apo-14 kDa showed an average of 55.7% homology to fish species belonging to Percomorpha, 42.0% homology to Japanese eel, and 37.2% homology to cypriniform species.

Teleosts possess divergent isoforms of apoA-I, apoA-IV, and apoE in the multigene subfamily. For example, there are four apoA-IV isoforms and two apoE isoforms in pufferfish (Kondo et al., 2005) and two apoA-I isoforms in rainbow trout (Delcuve et al., 1992). The presence of these diverse isoforms can be explained by whole-genome duplication(s) that occurred during the evolution of ray-finned fishes (Meyer and Schartl, 1999). However, only a single isoform each of apoA-I and apoE in barred knifejaw cDNA libraries were found despite our extensive sequencing efforts.

Multiple sequence alignments

ApoA-I is the major protein constituent of high-density lipoproteins (HDLs) and plays a crucial role in cellular cholesterol homeostasis through the reverse transport of cholesterol from tissues to the liver for excretion and regulation of its synthesis (Mahley et al., 1984; Lenich et al., 1988). More recent studies have indicated that this protein is also involved in a variety of defensive functions in fishes, including anti-viral, anti-microbial, and anti-inflammatory activities [see references in Villarroel et al. (2007)]. ApoE serves as a structural component for diverse classes of lipoproteins and plays an important role in recognition and uptake of lipoproteins from plasma and redistribution of cholesterol among tissues (Driscoll and Getz, 1984; Mahley et al., 1984; Rajavashisth et al., 1985; Lenich et al., 1988).

The secondary structures of barred knifejaw apoA-I and apoE consist of a signal peptide region, propeptide and N-terminal regions, a 33-AA unit (region 1-3), and 11- and 22-AA repeat units (regions 4 to 19) (Fig. 1a). Barred knifejaw apoA-I possesses a signal peptide that is 18 AA in length. This length is identical to other teleosts and mammals. The region 1-3 of barred knifejaw apoA-I has a 1-AA deletion at the alignment position 75, which is found in all other teleost fishes (alignment data not fully shown). It also comprises 32 AA residues instead of the common 33 AA in mammals. In addition, 1 AA is deleted in the 11 region and appears to be specific to barred knifejaw. The other 11- or 22-repeat units of barred knifejaw apoA-I are the same length as in other species. For apoE, the length of the signal peptide region is identical between teleosts and mammals (18 AA). A high degree of heterogeneity in the length of AA residues is observed in the regions 10 to 12, whereas the other repeat units are homologous. The deletion events in these regions are teleost lineage-specific. Thus, despite great sequence heterogeneity, the secondary structures of barred knifejaw apoA-I and apoE are well conserved with their teleostean and mammalian counterparts, especially in terms of the characteristic tandem repetitive units forming amphipathic α-helices. The signal peptide regions are highly homologous among them; however, the propeptide and N-terminal regions show the highest heterogeneity in sequence similarities and lengths.

Three apoC proteins (apoC-I to apoC-III) that comprise an apolipoprotein subfamily are the surface components of chylomicrons, very low-density lipoproteins (VLDLs), and HDLs. These apolipoproteins share a conserved genomic ORF structure and a 33-AA unit (Li et al., 1988) with similar distribution patterns. They also have similar molecular weights

and consistent purification yields (Jong et al., 1999). Among them, apoC-II is involved in lipoprotein metabolism and activates lipoprotein lipase (Mahley et al., 1984; Li et al., 1988). Figure 1b shows the AA alignment matrix of barred knifejaw apoC-II together with its teleostean and mammalian counterparts. The apoC-II comprises a signal peptide region, N-terminal region, 1-3 unit (region 1-3) and 22-AA repeat unit (region 4). Teleosts, including barred knifejaw, have a signal peptide region that is 20 AA in length. This region is shorter than the signal peptide region in mammals by 2 AA. The regions 1-3 and 4 are homologous among teleosts and mammals in terms of the length of AA residues, whereas the N-terminal region shows heterogeneity in length. The conserved region 4 is important for activation of lipoprotein lipase and metabolism of chylomicrons and VLDLs (Li et al., 1988). Barred knifejaw apoC-II exhibits a degree of homology not only with teleostean, but also with mammalian counterparts in terms of its secondary structure and AA length, but not its N-terminal region. In the present study, only apoC-II out of the three known apoC genes was found in barred knifejaw. A Blast search in GenBank indicated that zebrafish and gilthead seabream contained apoC-I homologs. However, there are no teleostean homologs to mammalian apoC-III.

In contrast to other apolipoprotein members identified from the wide range of vertebrate lineages, apo-14 kDa is fish-lineage specific and has a structure distinct from other apolipoproteins. Zhou et al. (2005) described an important role for apo-14 kDa in transport of yolk nutrients to the developing embryo and also in liver morphogenesis and growth during embryogenesis in orange-spotted grouper. A Blast search in GenBank revealed at least 11 teleostean homologs to barred knifejaw apo-14 kDa. Their AA alignment matrix is presented in Fig. 1c. The most likely cleavage sites for Percomorpha species, includeing barred knifejaw and Japanese eel, are between the aligned positions 20 and 21. In contrast, the most likely cleavage sites for Cypriniformes species are between the positions19 and 20. The former two taxa show a 1-AA deletion at the aligned position 21, whereas the latter taxon shows a 2-AA deletion at the positions 2 and 3. Japanese eel and cypriniforms are also distinguishable from Percomorpha species by an additional 3-AA deletion at the positions 70-72. Both the sequence alignment data and the analyses of cleavage sites indicate a clear differentiation between Percomorpha and Cypriniformes and an intermediate molecular feature in Japanese eel.

Molecular phylogenetic assumptions

Molecular phylogenetic trees of the three multigene apolipoprotein subfamilies were reconstructed using the NJ method. The NJ tree of apoA-I and apoE revealed the dichotomic branching pattern according to each encoded protein (Fig. 2a). The apoA-I branch was poorly supported; however, internal branches composed of teleostean and mammalian/avian taxa were strongly supported. Within the teleostean lineage, barred knifejaw formed a monophyletic group with European flounder, pufferfish, and gilthead seabream, which all belong to Percomorpha. In contrast, they were phylogenetically separate from fish species within Salmoniformes, Cypriniformes, and Anguilliformes. Meanwhile, apoE formed a monophyletic group with 100% strong bootstrap support in which teleosts and mammals were subdivided into two distinct lineages. In the teleostean lineage, zebrafish (NM_001020565) emerged at the primitive position, followed by two paraphyletic pufferfish isoforms (apoE-1 and apoE-2). Thereafter, zebrafish (two apoE isoforms), rainbow trout, and barred knifejaw displayed a moderate bootstrap value (85%).

The NJ tree of apoC-I, apoC-II, and apoC-III showed the trichotomic branching pattern according to each encoded protein (Fig. 2b). Within apoC-I and apoC-II branches, the internal branches composed of teleostean and mammalian taxa were strongly supported. The teleostean apoC-II lineage was monophyletic with moderate genetic affiliation to its mammalian counterpart. Within this lineage, rainbow trout, barred knifejaw, and gilthead seabream formed a monophyletic group with a relatively weak bootstrap value (62%).

The apo-14 kDa tree was composed of eight Percomorpha species, three cypriniforms, and one Japanese eel (Fig. 2c). As predicted by the AA sequence alignment matrix, Percomorpha and Cypriniformes formed each phylogenetically distinct monophyletic group, and Japanese eel was phylogenetically separated from these two taxa. Within the Percomorpha clade, barred knifejaw was phylogenetically affiliated with gilthead seabream.

The presence of homologous genomic structures (exon/intron organization) and tandemly repeating units suggests that apolipoprotein multigene family members originated from gene duplications and modify cations from the ancestral 11-nucleotide unit and therefore have a common evolutionary origin (Boguski et al., 1985; Rajavashisth et al., 1985; Li et al., 1988; Kondo et al., 2005). The apolipoprotein members of apoA-I, apoA-IV, and apoE diverged

Fig. 1. Alignment matrices of amino acid sequences of apolipoprotein A-I (apoA-I) and apoE (a), apoC-II (b), and apo-14 kDa (c) of barred knifejaw, *Oplegnathus fasciatus*, with their teleostean and mammalian orthologs. Boundaries of signal peptide (signal), propeptide (pro), N-terminal region (N-term), repeated structures (regions 1-3, 4 to 15) are indicated above the sequence matrix with arrow heads (▼) with inference to Kondo et al. (2005). Dots indicate identical amino acid residues compared to barred knifejaw sequences. Gaps are indicated by dashes inserted to optimize the alignment.

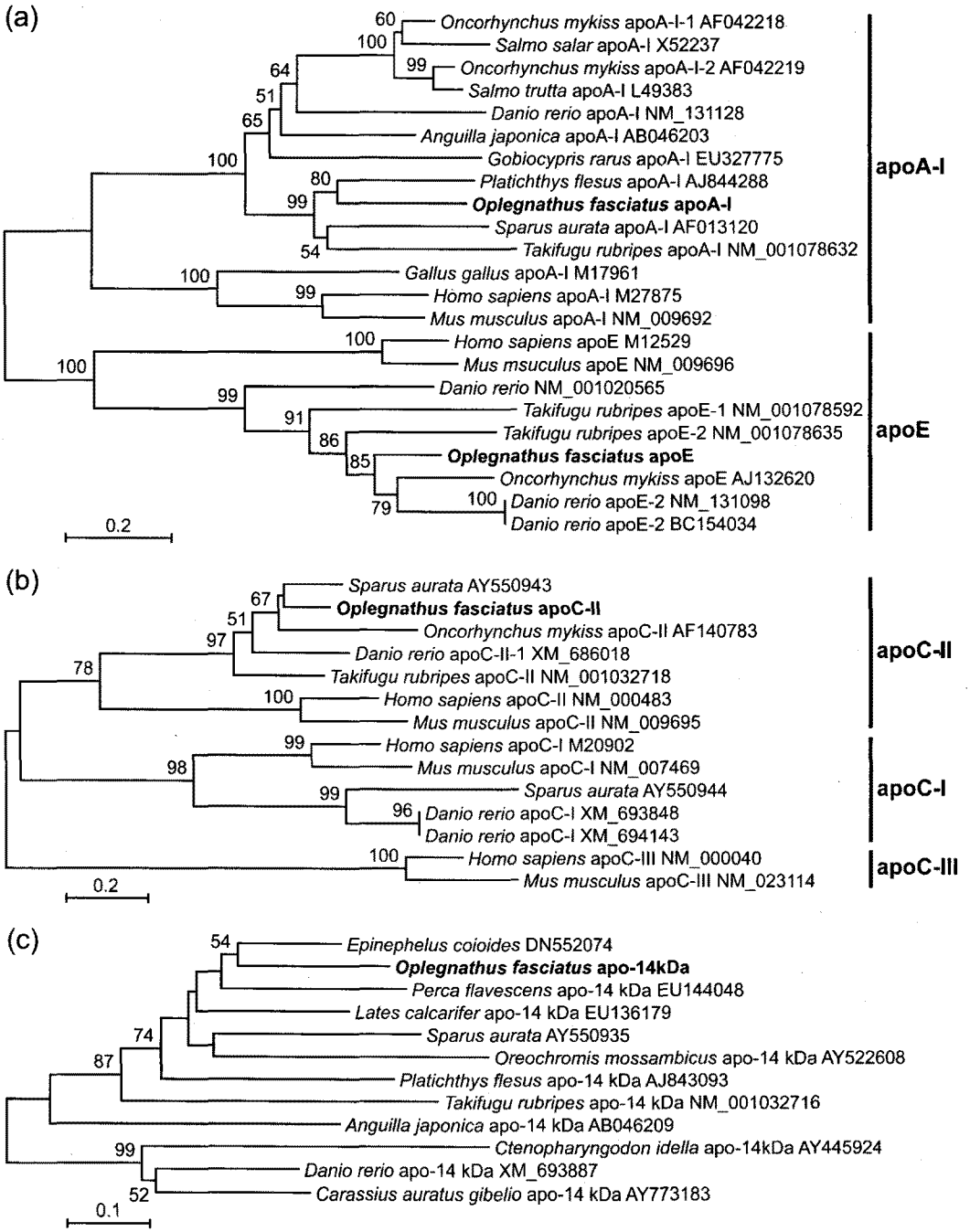

Fig. 2. Unrooted neighbor-joining trees inferred from amino acid sequences of apolipoprotein A-I (apoA-I) and apoE (a), apoC-I-III (b), and apo-14 kDa (c) of barred knifejaw, *Oplegnathus fasciatus*, with their teleostean and mammalian/avian homologs. The number at each branch node indicates the bootstrap value above 50%. ApoA-I, apoE, apoC-II, and apo-14 kDa of barred knifejaw analyzed in this study are in boldface.

following the most recent gene duplication events. It is believed that apoE evolved first and apoA-I and apoA-IV diverged thereafter by gene duplication and modi-fications (Boguski et al., 1985; Li et al., 1988). However, our results do not confidently support this evolutionary history. Instead, our phylogenetic tree suggests that the common ancestor before the split of the Actinopterygii (ray-finned fishes) and Sarcopterygii (tetrapods) would have possessed the two protein-encoding genes. This phylogenetic assumption is also supported in the NJ tree of the apolipoprotein C subfamily.

Tissue distribution of apolipoprotein transcripts

The four apolipoprotein genes of barred knifejaw juveniles showed tissue-specific patterns of mRNA expression among ten somatic tissues examined (Fig. 3). Barred knifejaw apoA-I transcripts were highly expressed in the liver, intestines, and brain. Expression

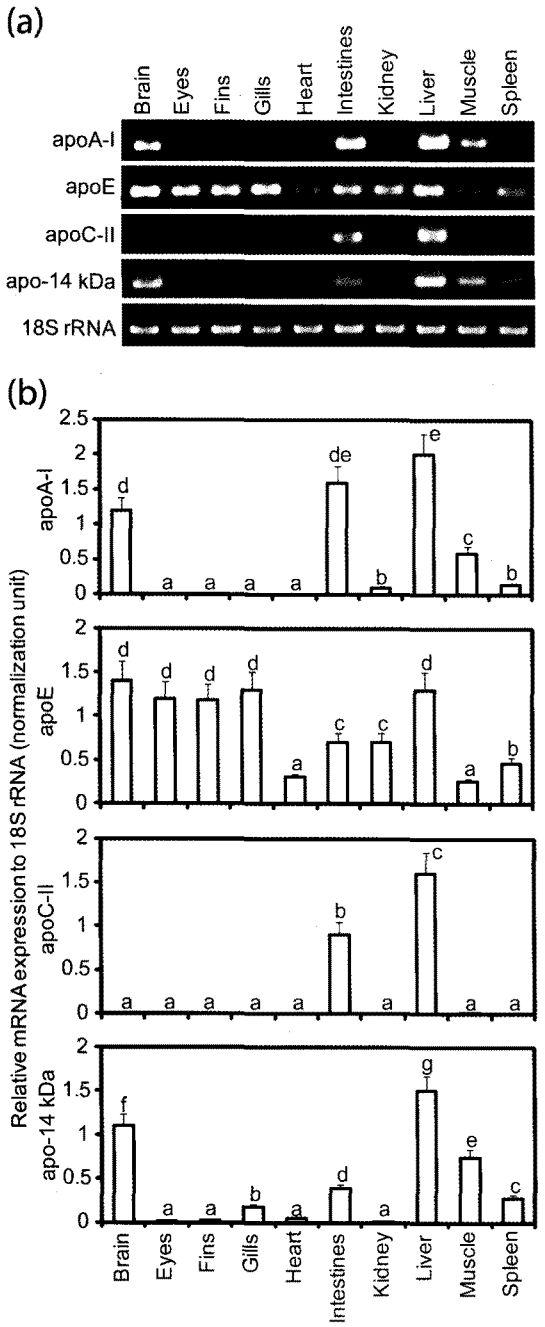

Fig. 3. Tissue distributions of four apolipoprotein mRNAs in barred knifejaw, *Oplegnathus fasciatus*. RT-PCR was carried out with 1 μL of RT-product (cDNA) as a template for PCR amplification using a primer pair each specific to one of barred knifejaw apolipoprotein cDNAs (see Table 1 for primer names and sequences) from brain, eyes, fins, gills, heart, intestines, kidney, liver, muscle, and spleen. 18S rRNA was used as an internal control for normalizing apolipoprotein transcripts. (a) Representative EtBr-stained gels showing apolipoprotein transcripts amplified by semi-quantitative RT-PCR. (b) Histograms (means based on triplicate assays) showing the relative expression rates of apolipoprotein transcripts. Standard deviations are indicated by T-bars. Signal intensities (INT/mm^2) of the bands are assigned using the Quantity-One^{TM} image analysis software, implemented in VersaDoc 4000 (Bio-Rad, USA). Means with the same letters on each histogram are not statistically different based on an analysis of variance (ANOVA) followed by Duncan's multiple range tests at P=0.05.

was moderate in the muscle, and weak in the kidney and spleen. Expression was barely detectable in the eyes, fins, gills, and heart (P<0.05). ApoE mRNAs were ubiquitously expressed across all tissues, although expression levels varied. Expression of apoC-II mRNAs was predominant in the liver and intestines (P<0.05). Barring a very weak RT-PCR signal in the muscle, apoC-II mRNAs were not detected in the other remaining tissues. Finally, apo-14 kDa exhibited a similar pattern of mRNA expression as apoA-I; the liver, brain, muscle, intestines, spleen, and gills all displayed high or moderate expression compared to the other tissues (P<0.05).

The liver and intestines are the major source of plasma apolipoproteins (Li et al., 1988), and our semi-quantitative RT-PCR assay detected all four barred knifejaw apolipoprotein gene transcripts in those tissues. Most vertebrates investigated, including fish species, predominantly express apoA-I transcripts in the liver and intestines (Mahley et al., 1984; Lenich et al., 1988; Powell et al., 1991; Delcuve et al., 1992; Llewellyn et al., 1998; Kondo et al., 2001, 2005). However, our data show that mRNA expression of barred knifejaw apoA-I is not restricted to those two tissues; apoA-I transcripts are also found in other tissues and demonstrate considerably high levels in the brain. Relatively high expression levels of the apoA-I gene in the fish brain may be explained by its involvement in optic nerve regeneration (Harel et al., 1989). The more or less ubiquitous expression of apoA-I transcripts has only been reported in one other fish species, mud loach (Lee et al., 2007). On the other hand, a wide tissue distribution of apoA-I transcripts has been reported in chickens (Byrnes et al., 1987; Rajavashisth et al., 1987).

The most striking difference between barred

knifejaw apoE and previous findings on fish apoEs (e.g., Kondo et al., 2005) is the ubiquitous expression across all somatic tissues. For example, two pufferfish apoE isoforms showed mRNA expression only in the intestines for both isoforms and the brain for apoE-2. On the other hand, apoE transcripts have been detected in a variety of mammalian tissues (Driscoll and Getz, 1984; Lenich et al., 1988). Each apolipoprotein gene (i.e., apoA-I, apoA-IV, and apoE) consists of multiple isoforms in the fish genome. These multiple isoforms may have distinct patterns of tissue-specific expression, which conesquently confer a unique role for each isoform. Further examination of these diverse isoform sequences will be valuable for better elucidating their tissue-specific distributions and functions. We also demonstrated that barred knifejaw apoC-II shows robust expression patterns in the liver and intestines. These data are consistent with previous studies on mammals (Myklebost et al., 1984; Lenich et al., 1988; Hoffer et al., 1993) and fishes (Shen et al., 2000; Kondo et al., 2005), which reported that the liver and intestine should be the major sites of apoC-II protein synthesis. The synthesis of apo-14 kDa mRNAs occurred mostly in the liver and brain of barred knifejaw. These data are in general agreement with results from other fish species (Kondo et al., 2001, 2005; Zhou et al., 2005); however, apo-14 kDa transcripts were also moderately expressed in several tissues.

In summary, the genetic determinants of four apolipoprotein members were isolated and characterized from barred knifejaw. The results obtained from sequence characterization, molecular phylogenies and mRNA tissue distribution assays indicate that the structural properties and expression patterns of apolipoproteins are complex and species-specific depending on each apolipoprotein member in the teleost lineage. Additionally, these patterns are distinct from their mammalian counterparts.

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