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Short communication

Analysis of *apolipoprotein* multigene family in spotted sea bass (*Lateolabrax maculatus*) and their expression profiles in response to *Vibrio harveyi* infection

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ABSTRACT

Apolipoproteins (Apos), which are the protein components of plasma lipoproteins, play important roles in lipid transport in vertebrates. It has been demonstrated that in teleosts, several Apos display antimicrobial activity and play crucial roles in innate immunity. Despite their importance, *apo* genes have not been systematically characterized in many aquaculture fish species. In our study, a complete set of 23 *apo* genes was identified and annotated from spotted sea bass (*Lateolabrax maculatus*). Phylogenetic and homology analyses provided evidence for their annotation and evolutionary relationships. To investigate their potential roles in the immune response, the expression patterns of 23 *apo* genes were determined in the liver and intestine by qRT-PCR after *Vibrio harveyi* infection. After infection, a total of 20 differentially expressed *apo* genes were observed, and their expression profiles varied among the genes and tissues. 5 *apo* genes (*apoA1*, *apoA4a.1*, *apoC2, apoF* and *apoO*) were dramatically induced or suppressed (log2 fold change > 4, $P < 0.05$), suggesting their involvement in the immune response of spotted sea bass. Our study provides a valuable foundation for future studies aimed at uncovering the specific roles of each *apo* gene during bacterial infection in spotted sea bass and other teleost species.

1. Introduction

Apolipoproteins (Apos), the protein components of various plasma lipoproteins, play important roles in lipid transport and uptake through the circulation system in vertebrates [1,2]. Apos are grouped into classes of exchangeable (*ApoA*, *ApoC* and *ApoE* classes) and non-exchangeable Apos (*ApoB* classes) [3]. Exchangeable Apos, relatively smaller in size and characterised by repeated amphipathic α helix regions, are soluble in aqueous environments [4,5]. In contrast, non-exchangeable Apos are one of the largest monomeric proteins that are made up of amphipathic β strands as lipid-associating motifs and are highly insoluble in aqueous solution [6]. Exchangeable Apos are capable of moving from one lipoprotein particle to another as opposed to the non-exchangeable Apos that remain with one lipoprotein particle from biosynthesis to catabolism [4]. Additionally, several Apos including ApoD, ApoF, ApoH, ApoL, ApoM, and ApoO classes, have been discovered and are regarded as atypical apolipoproteins based on their primary structures. The structures of atypical apolipoproteins show no similarity to exchangeable or non-exchangeable apolipoproteins, but they are also components of lipoprotein and are associated with lipid metabolism [7,8].

Apos have been extensively studied in humans (*Homo sapiens*), and a relationship between specific lipoproteins and cardiovascular disease is apparent [9,10]. Instead of using carbohydrates, fish always utilize lipids as the main energy source [11], suggesting that lipid metabolism and lipoprotein physiology may be more important for homeostasis in fish than it is in human [12]. Although studies about Apos in lower vertebrates remain limited, accumulating evidence supports the hypothesis that Apos are involved in immune responses in teleosts. For example, *ApoA1* and *ApoA2* in common carp (*Cyprinus carpio*) display antimicrobial activity in blood and play crucial roles in innate immunity [13]. Similar functions of *ApoA1* against bacteria have also been discovered in rainbow trout (*Oncorhynchus mykiss*) [14] and orange-spotted grouper (*Epinephelus coioides*) [15]. The protein profile of *ApoA1*, *ApoA2* and *Apo*-14kDa in mucus was found to be significantly changed in Atlantic salmon (*Salmo salar*) after infection by

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Lepeophtheirus salmonis and *Vibrio anguillarum* [16]. In channel catfish (*Ictalurus punctatus*), a series of *apo* genes, including *apoBa*, *apoDb*, *apoEa*, *apoF*, *apoL4*, *apoOL* and *apo*-14kDa, was found to be differentially expressed after *Edwardsiella ictaluri* and *Flavobacterium columnare* infection [17]. These studies suggest that *apo* genes are potential immune modulators participating in the immune response and that the functions of *apo* genes may vary among different teleost species.

Spotted sea bass (*Lateolabrax maculatus*) are considered one of the most economically important and promising aquaculture species in East Asia. However, the spotted sea bass industry has been greatly hindered by bacterial infection diseases [18,19], particularly by *Vibrio harveyi*, which is recognized as one of the most serious pathogens for marine fishes and invertebrates [20]. To design effective strategies for disease management, it is of great significance to identify immunity-related genes and understand their functions in response to bacterial challenges. However, *apo* genes have not been systematically characterized in spotted sea bass. In this study, a total of 23 *apo* genes were identified and characterized in spotted sea bass. Phylogenetic and homology analyses were conducted to confirm their annotation and investigate their evolutionary relationships. To provide insight into the function of the *apo* genes of spotted sea bass in immune responses, their mRNA expression patterns were determined after *V. harveyi* infection.

2. Materials and methods

2.1. Ethics statement

Ethics approval for this study was obtained from the Institutional Review Board at Ocean University of China (Permit Number: 20141201) and all participants provided written informed consent. This study was not involved in endangered or protected species and experiments were performed in accordance with relevant guidelines.

2.2. Genome-wide identification and sequence analyses

To identify *apo* genes in spotted sea bass, TBLASTN was used to search the reference genome database (PRJNA408177), Iso-Seq database (SRX5260407), and RNA-Seq databases (SRR4409341, SRR4409397) using the *apo* full-length amino acid sequences from human, mouse (*Mus musculus*), zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), and torafugu (*Takifugu rubripes*) retrieved from NCBI, Ensemble and UniProt databases as queries with e-values of 1e-5. Multiple alignment was conducted with ClustalW and the redundant sequence was then removed. Tandem arrangement genes were identified by their locations in the reference genome. Gene structures were predicted by the Softberry program (http://linux1.softberry.com/) based on the zebrafish model, and open reading frames were identified by the ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/). In addition, the molecular weight (MW) and theoretical isoelectric point (pI) of apolipoproteins was characterized using the online ProtParam tool (https://web.expasy.org/protparam/).

2.3. Phylogenetic and collinear analyses

To investigate the evolutionary relationship of the *apo* multigene family, a phylogenetic tree was constructed using the amino acid sequences of *apo* genes of several representative mammals and teleosts downloaded from NCBI and Ensemble databases, including human, mouse, zebrafish, channel catfish, torafugu, tilapia (*Oreochromis niloticus*), and medaka. Multiple protein sequences were aligned by MUSCLE with default parameters. The phylogenetic tree was built using MEGA 7.0 software based on the neighbour-joining (NJ) method and Jones-Taylor-Thornton model with 1000 bootstrap replicates [21,22]. The phylogenetic tree was further modified by the online iTOL website (https://itol.embl.de/).

To compare the chromosome distribution of *apo* genes among zebrafish, tilapia and spotted sea bass, their reference genome information was downloaded from the NCBI database, and their homology relationships were constructed based on their amino acid sequences and their chromosome location by the Circos program (v0.69). To provide additional evidence for the annotation of *apo* genes, a syntenic analysis was conducted by comparing neighbouring genes in spotted sea bass with zebrafish and tilapia. The conserved syntenic regions among the three teleosts were determined by NCBI and Genomicus databases (v95.01).

2.4. Bacterial challenge experiment

One-year-old spotted sea bass (body length: 48.76 ± 4.26 cm, body weight: 178.25 ± 18.56 g) were acclimated at Shuangying Aquaculture Company, Dongying, Shandong, China for one week. *V. harveyi* was cultured from a single colony, re-isolated from a symptomatic fish and biochemically confirmed before being inoculated in brain heart infusion broth and incubated in a shaker incubator overnight. The concentration of the bacteria was determined using colony forming units (CFU)/mL by plating 10 μl of 10-fold serial dilutions onto BHI agar plates. After acclimation, individual fish were treated by intraperitoneal injection of 0.1 ml *V. harveyi* solution at a concentration of 3×10^6 CFU/mL. One fish group injected with the same volume of PBS served as the control group. The individuals were challenged in 120 L square tanks in triplicate at a density of 30 fish per tank. During the entire experiment, the water temperature (18 ± 0.5 °C), pH (7.8–8.15), salinity (30–33 ppm) and dissolved oxygen (6.7–7.5 mg/L) remained stable. Three individuals per tank were euthanized with MS-222 (300 mg/L). Liver and proximal intestine tissues were sampled at each time point, including 0 h, 6 h, 12 h, 24 h, 48 h, and 72 h after bacterial challenge. Then, the samples were flash frozen in liquid nitrogen and stored at −80 °C until RNA extraction.

2.5. RNA extraction and quantitative real-time PCR analyses (qRT-PCR)

Total RNA from experimental samples was extracted using TRIzol reagent (Invitrogen, CA, USA) according to the manufacturer's protocol. The RNA concentration and quality were assessed by the ratio of OD260/OD280 using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The integrity of RNA was evaluated by 1% agarose gel electrophoresis [23]. To minimize the variation among individuals, RNA of each fish from same tank were equally pooled together. Then, pooled RNA was reverse transcribed to cDNA using the PrimeScriptTM RT reagent kit (Takara, Otsu, Japan), and the concentration of cDNA products was adjusted to 500 ng/μl for subsequent qRT-PCR experiments. Specific primers were designed by Primer 5 software based on conserved regions of the *apo* genes in spotted sea bass (Supplementary Table 1). The 18S rRNA was set as the internal reference gene based on a previous reference gene expression validation study of spotted sea bass [24]. cDNA (10 × diluted) was used as the template for qRT-PCR, and the SYBR Premix Ex TaqTM kit was used for qRT-PCR (Takara, Shiga, Japan). qRT-PCR experiments were performed on Applied Biosystems 7300 machines (Applied Biosystems, CA, USA). The PCR volume was 20 μl, including 2 μl of cDNA, 10 μl of SYBR premix Ex Taq, 0.4 μl of forward primers, 0.4 μl of reverse primers, 0.4 μl of ROX Reference Dye, and 6.8 μl of ddH_2O. The PCR analysis was repeated in triplicate (technical replicates), and PCR amplification was carried out using the following conditions: 95 °C for 30 s and 40 cycles of 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 30 s. Cycle threshold (Ct) values generated by qRT-PCR were used to calculate the relative expression fold change by the $2^{-\Delta\Delta Ct}$ method. The statistical analysis was performed using SPSS 17.0 software (SPSS Inc., Chicago, USA). The means of the relative mRNA expression level were assessed by one-way ANOVA followed by Duncan's multiple tests. The differences were considered as statistically significant when the P-value < 0.05. The relative fold change of expression levels was showed using

Table 1
Summary of *apo* genes in spotted sea bass.

Gene name	Chromosome ID	protein length (aa)	classify	MW (kDa)	pI	NCBI accession number
apoA1a	Chr5	266	exchangeable	29.23	5.23	MK599340
apoA4a.1	Chr16	256	exchangeable	28.97	4.77	MK599341
apoA4a.2	Chr16	257	exchangeable	29.33	5.05	MK599342
apoA4b	Chr7	255	exchangeable	28.76	4.72	MK599343
apoBa	Chr19	4415	Non-exchangeable	496.31	5.68	MK599344
apoBb.1	Chr18	3818	Non-exchangeable	424.56	5.23	MK599345
apoBb.2	Chr18	4828	Non-exchangeable	530.94	6.25	MK599346
apoC1	Chr7	87	exchangeable	9.63	5.32	MK599347
apoC2	Chr7	97	exchangeable	10.85	5.02	MK599348
apoDa.1	Chr10	201	atypical	22.03	4.93	MK599349
apoDa.2	Chr10	190	atypical	20.85	5.09	MK599350
apoDa.3	Chr10	186	atypical	21.17	8.88	MK599351
apoDb.1	Chr20	201	atypical	22.34	5.18	MK599352
apoDb.2	Chr20	189	atypical	21.05	7.58	MK599353
apoEa	Chr16	262	exchangeable	30.49	5.24	MK599354
apoEb	Chr7	276	exchangeable	31.16	4.95	MK599355
apoF	Chr4	442	atypical	47.32	5.27	MK599356
apoL1	Chr10	391	atypical	42.83	4.99	MK599357
apoL4	Chr15	568	atypical	62.45	5.32	MK599358
apoM	Chr7	189	atypical	21.92	5.12	MK599359
apoO	Chr10	198	atypical	20.85	8.87	MK599360
apoOL	Chr14	497	atypical	29.10	9.57	MK599361
*apo14*kDa	Chr7	143	atypical	15.71	5.68	MK599339

MW: the molecular weight of apolipoprotein; pI: isoelectric point of apolipoprotein.

Log_2 Fold Chang (log_2FC) and these histogram figures were made in SigmaPlot 12.5 software (Systat Software Inc., San Jose, USA).

3. Results and discussion

3.1. Identification and annotation of apo genes in spotted sea bass

In our study, a total of 23 *apo* genes were identified from the genome and transcriptome databases of spotted sea bass. The characteristics of the spotted sea bass *apo* genes are summarized in Table 1, and their cDNA sequences have been submitted to the GenBank database (Table 1). In detail, the lengths of the predicted protein sequences ranged from 87 to 4828 amino acids, the molecular weights varied from 9.63 to 530.94 kDa, and the pIs were between 4.72 and 9.58. Based on published literature and their mode of action, the 23 *apo* genes were predominantly grouped into the 2 major types: exchangeable (*apoA1a*, *apoA4a.1*, *apoA4a.2*, *apoA4b*, *apoC1*, *apoC2*, *apoEa*, and *apoEb*) and non-exchangeable (*apoBa*, *apoBb.1* and *apoBb.2*). The remaining *apo* genes without clear classification in the literature were regarded as atypical (*apoDa.1*, *apoDa.2*, *apoDa.3*, *apoDb.1*, *apoDb.2*, *apoF*, *apoL1*, *apoL4*, *apoM*, *apoO*, *apoOL*, and *apo14-kDa*).

3.2. Phylogenetic and homology analyses

To confirm the annotation and investigate the phylogenetic relationships of *apo* genes, the predicted amino acid sequences of spotted sea bass and several selected species' *apo* genes were used to construct a phylogenetic tree. As shown in Fig. 1, the *apo* genes of spotted sea bass were clustered with their counterparts in selected teleosts as expected, and 3 groups (exchangeable, non-exchangeable and atypical) were generated. The tree provided strong evidence for the annotation and classification of *apo* genes in spotted sea bass. It was clear that the sizes of the non-exchangeable apolipoproteins in these tested species were significantly larger than those of the exchangeable or atypical apolipoproteins (Fig. 1).

To further investigate duplicated *apo* genes and compare their genomic distribution in teleosts, gene homology relationships of zebrafish, tilapia and spotted sea bass were constructed (Fig. 2A). The *apo* genes in spotted sea bass were dispersed among 10 chromosomes, including chr4, chr5, chr7, ch10, chr14, chr15, chr16, chr18, chr19 and chr20 (Fig. 2A). The homology analysis in zebrafish, tilapia and spotted sea bass showed that *apo* genes were distributed in clusters or individually, and 4 *apo* gene clusters of spotted sea bass were located on chr7, chr10, chr16 and chr18 (Fig. 2A).

A syntenic analysis was performed to clearly delineate the distribution of *apo* genes in spotted sea bass. As shown in Fig. 2B, the genomic distribution of *apo* genes was classified into 2 types (individual or tandem arrangements). Seven *apo* genes (*apoBa*, *apoF*, *apoL1*, *apoL4*, *apoM*, *apoOb*, and *apoOL*) were separately arranged on chr19, chr4, chr10, chr15, chr7 chr20 and chr14 (Supplementary Fig. 1). In contrast, six *apo* genes (*apoA4b*, *apoA1*, *apoEb*, *apoC1*, *apo*-14kDa, and *apoC2*), five of which belonged to the exchangeable group, were tandemly arranged on chr7 of the spotted sea bass genome (Fig. 2B). In addition, tandem arrangement events were also observed for *apoA4a*, *apoBb*, and *apoDa* (Fig. 2B).

In the overall syntenic analysis (Fig. 2B), *apo* genes in spotted sea bass shared a conserved genomic neighbourhood compared with those of zebrafish and tilapia, which provides additional supporting evidence for the annotation of *apo* genes in spotted sea bass. The duplication or cluster formation of the *apo* gene may be the result of teleost-specific whole-genome duplication, recombination events or independent tandem duplications [25,26]. The cluster distribution of *apo* genes is considered a clue to the evolution of *apo* genes.

3.3. Copy numbers of apo genes

The copy numbers of *apo* genes in spotted sea bass and several representative vertebrates are summarized in Table 2. Overall, the number of *apo* genes varied greatly among selected vertebrates, ranging from 10 to 29. For example, 22 *apo* genes have been identified in human, while only 10 *apo* genes have been found in chickens. A total of 19–25 *apo* genes have been identified in teleost fishes. The *apoA5*, *apoC3*, *apoC4*, *apoH*, *apoL5*, *apoL7*, *apoL8*, *apoL9*, *apoL10*, *apoL11*, and *apoN* genes have been found to be absent in teleost genomes. In contrast, *apo-14kDa* is a teleost-specific *apo* gene, which has not yet been discovered in any genomes of higher vertebrates [12,27]. Previous studies stated that fish *apo-14kDa* is the homologue of mammalian *apoA2,* although fish *apo-14kDa* lacks a propeptide and contains more internal repeats than mammalian *apoA2* [28]. Additionally, no *apo* gene duplications have been observed in higher vertebrates, except for a few

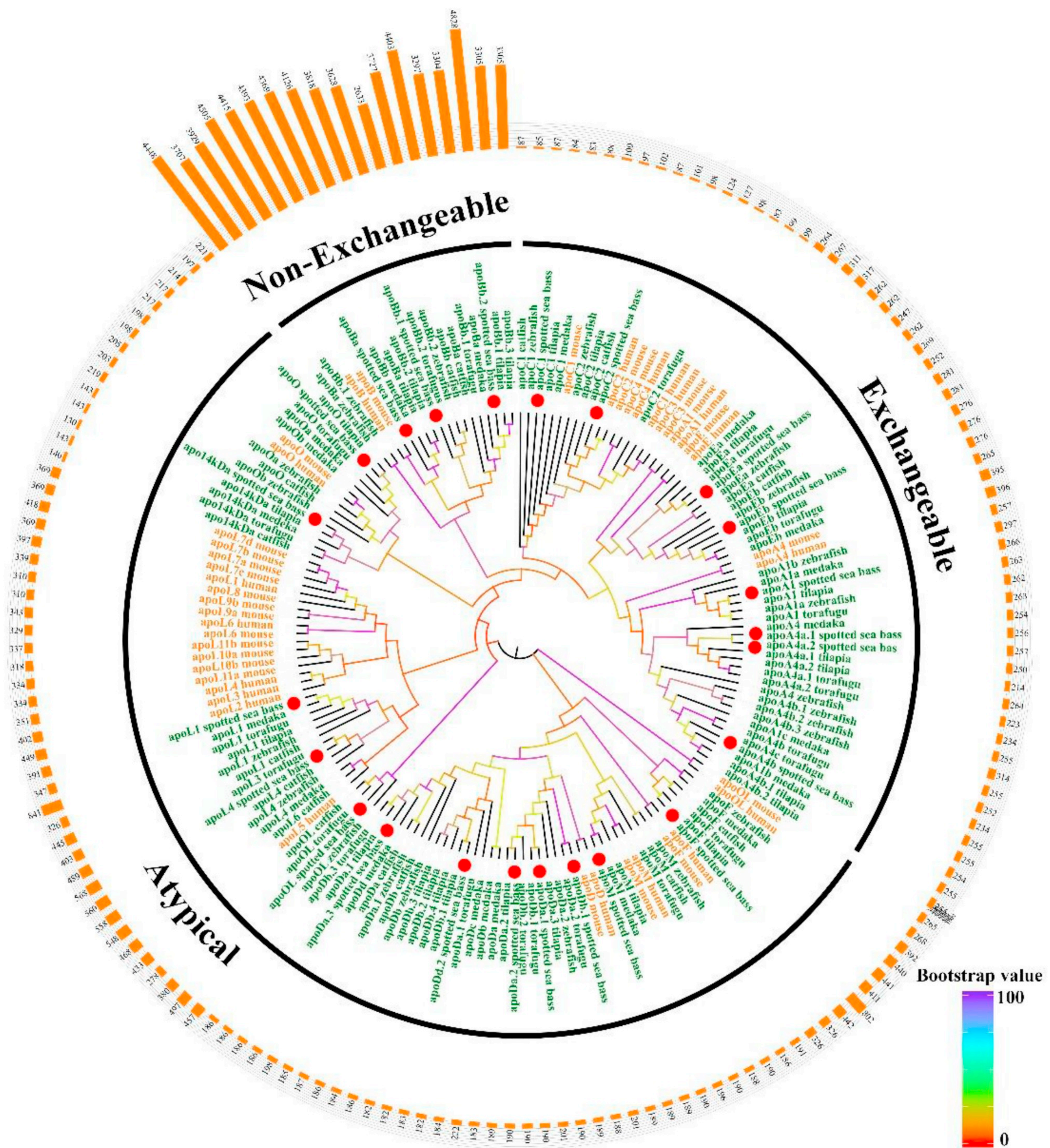

Fig. 1. Phylogenetic relationships of *apo* genes in spotted sea bass and selected vertebrate species. The *apo* genes of higher vertebrates were marked with yellow text and the *apo* genes of teleosts was green. The bootstrap values were represented by various colors and *apo* genes of spotted sea bass were labeled with red dots. The orange bars outside phylogenetic tree represented the size of deduced amino acid of these genes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

apoL genes in mouse. However, duplicated copies, such as *apoA1*, *apoA4*, *apoB*, *apoD*, and *apoE*, which are likely derived from teleost-specific whole genome duplication, homologous recombination or other evolutionary processes, are commonly found in teleosts [17,29].

3.4. Expression analysis of apolipoprotein genes after V. harveyi infection

Recently, accumulating evidence has revealed that Apos are synthesized mainly in the liver and intestine [1,30]. The liver and intestine in vertebrates are not only the major target sites for lipid uptake and synthesis but also the primary organs responsible for innate immunity [2,31,32]. In the present study, qRT-PCR analysis was conducted to determine the expression profiles of a complete set of *apo* genes in liver and intestine tissues at 0 h, 12 h, 24 h, 48 h and 72 h after *V. harveyi* infection. A total of 20 differentially expressed *apo* genes were observed, and their expression profiles varied among the genes and tissues. The expression of 5 *apo* genes (*apoA1*, *apoA4a.1*, *apoC2, apoF* and *apoO*) were dramatically induced or suppressed ($\log_2$FC > 4, $P < 0.05$).

In the liver, the expression of 14 of 23 *apo* genes was significantly

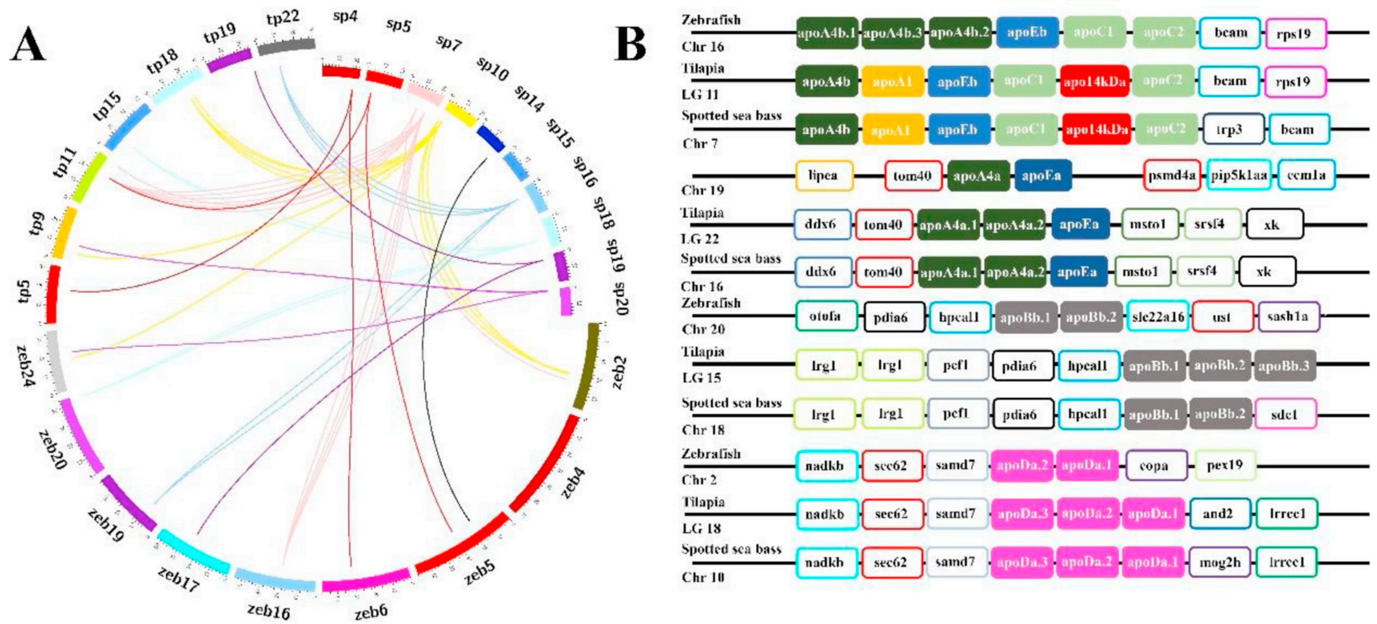

Fig. 2. Homologous relationships of *apo* genes in zebrafish, tilapia and spotted sea bass (A) Chromosome distribution of orthologous *apo* genes in zebrafish, tilapia and spotted sea bass. Zeb, tp and sp represent zebrafish, tilapia and spotted sea bass, respectively. Links show the homologous genes among the species. The chromosome units were set as 25 Mb. **(B) Syntenic analysis of *apo* clusters in zebrafish, tilapia and spotted sea bass.** The *apo* genes are marked with colour-filled boxes. The full names of the genes are listed in Supplementary Table 2. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
The copy number of *apo* genes in several representative mammals and teleosts.

Name	human	mouse	rabbit	chicken	frog	zebrafish	catfish	tilapia	Altantic salmon	torafugu	medaka	spotted sea bass
apoA1	1	1	1	1	1	2	2	1	2	1	3	1
apoA2	1	1	1	1	0	1	0	0	0	0	0	0
apoA4	1	1	1	1	1	4	1	4	1	4	1	3
apoA5	1	1	1	1	1	0	0	0	0	0	0	0
apoB	1	1	1	1	1	3	2	4	5	2	2	3
apoC1	1	1	1	0	1	1	1	1	1	0	1	1
apoC2	1	1	1	0	1	1	1	1	1	1	0	1
apoC3	1	1	1	1	0	0	0	0	0	0	0	0
apoC4	1	1	1	0	0	0	0	0	0	0	0	0
apoD	1	1	1	1	0	3	2	7	2	5	4	5
apoE	1	1	1	0	1	2	2	2	3	2	2	2
apoF	1	1	1	0	1	1	1	1	1	1	1	1
apoH	1	1	1	1	1	0	0	0	0	0	0	0
apoL1	1	0	0	0	0	1	1	1	1	1	0	1
apoL2	1	0	1	0	0	0	0	0	0	0	0	0
apoL3	1	0	1	0	0	0	0	0	1	1	0	0
apoL4	1	0	0	0	0	1	1	0	0	0	1	1
apoL5	1	0	0	0	0	0	0	0	0	0	0	0
apoL6	1	1	0	0	0	0	1	0	0	0	0	0
apoL7	0	4	0	0	0	0	0	0	0	0	0	0
apoL8	0	1	0	0	0	0	0	0	0	0	0	0
apoL9	0	2	0	0	0	0	0	0	0	0	0	0
apoL10	0	2	0	0	0	0	0	0	0	0	0	0
apoL11	0	2	0	0	0	0	0	0	0	0	0	0
apoM	1	1	1	0	1	1	1	1	1	1	1	1
apoN	0	1	0	0	0	0	0	0	0	0	0	0
apoO	1	1	1	1	1	2	1	1	1	1	2	1
apoO-like	1	1	1	1	1	1	1	0	1	1	1	1
apo-14kDa	0	0	0	0	0	0	1	1	0	1	1	1
Total	22	29	18	10	12	25	19	25	21	22	20	23

Yellow color represented the duplicated *apo* genes in teleosts. Gray color showed the absence of *apo* genes in Teleost. Teleost-specific *apo*-14kDa was marked with red color. Human-specific or mouse-specific *apo* genes were marked with green color.

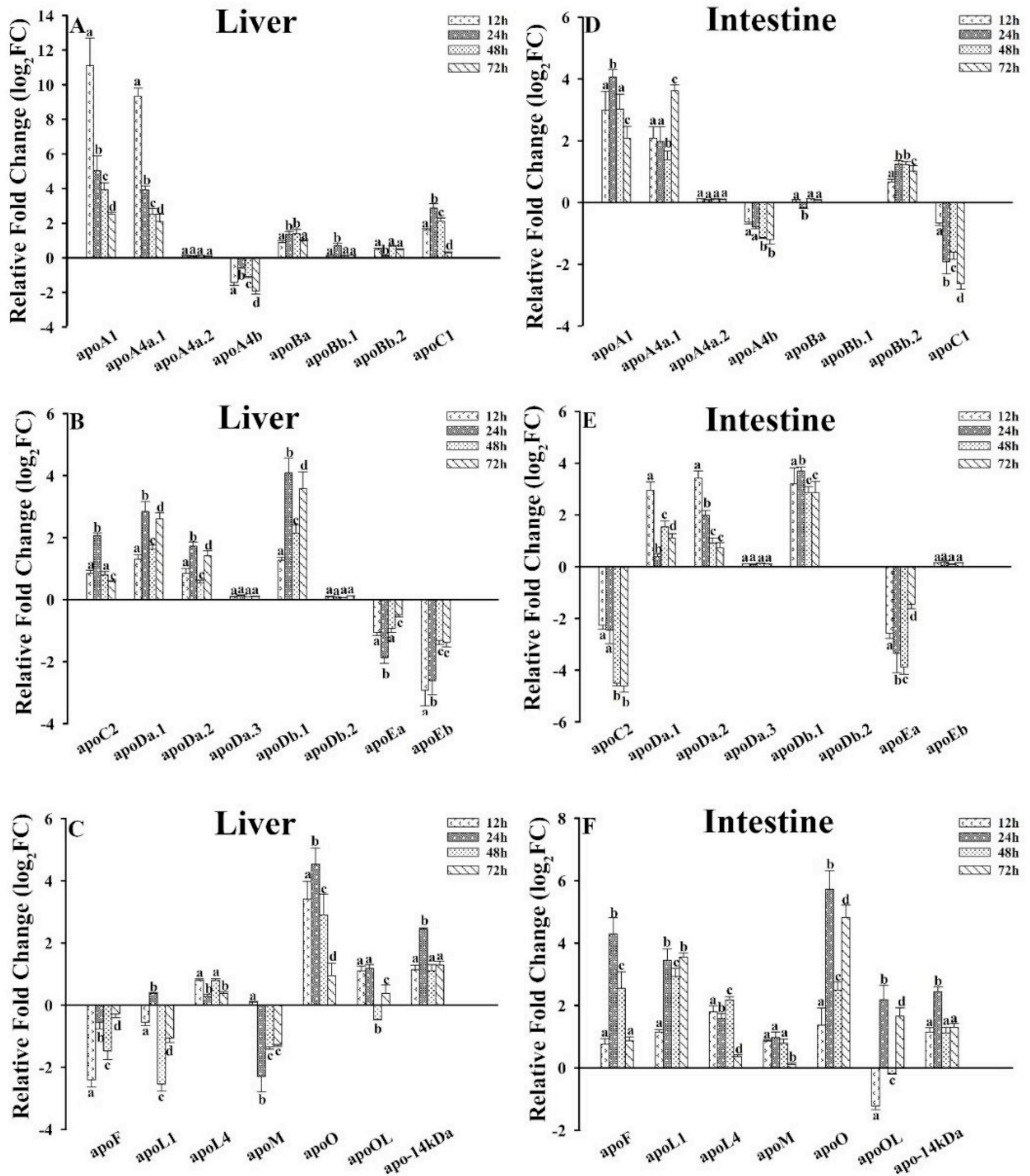

Fig. 3. Expression patterns of 23 *apo* genes in the liver and intestine of spotted sea bass at 0h, 12h, 24h, 48h and 72h after *V. harveyi* infection. Gene expression patterns were presented as fold change relative to control samples (0 h). Y-axis indicated the mRNA relative expression after logarithm 2 based transformation. Various letters among same tissue and gene indicated significant difference ($P < 0.05$).

induced after *V. harveyi* infection, while the expression of 6 *apo* genes was suppressed (Fig. 3A–C), suggesting their roles in the immune response, particularly the genes with large expression fold-changes. Notably, *apoA1* and *apoA4a.1* were the most highly up-regulated genes in the liver at 12 h post-infection, with log_2FC as 11.1- and 9.3, respectively (Fig. 3A). The antibacterial activity of *ApoA1* has been proven in several teleosts, including carp [13], rainbow trout [14], striped bass (*Morone saxatilis*) [33], channel catfish [34] and grouper (*Epinephelus*

sp.) [15]. More specifically, *ApoA1* forms cationic amphipathic α-helices, intercalates into lipid bilayers, neutralizes the major pathogenic factor LPS, and inhibits inflammatory cytokines *in vitro* [35–38]. *ApoA4a.1* displays a similar cationic amphipathic α-helical structure to *ApoA1*, which may also exhibit antibacterial capacity. In contrast, *apoA4b* was significantly down-regulated at 12 h post-infection (Fig. 3A). The *apoA4b* orthologue in gilthead sea bream (*Sparus aurata*) also acts as a negative regulator in response to bacterial infection [39]. It has been demonstrated that down-regulation of *apoA4b* in pig (*Sus scrofa*) is caused by the cytokine interleukin-1 [40]. The genes *apoBa* and *apoBb.2* were differentially expressed at 12 h after infection (Fig. 3A). Accumulating evidence indicates that *ApoB*, which is the main protein component of VLDL and LDL, can bind and inhibit cytokine release to modulate the functions of the host innate immune system [41,42]. *apoDa.1*, *apoDa.2* and *apoDb.1* showed increased expression profiles in the liver at 12 h after infection, and their expression level peaked at 24 h, especially *apoDb.1,* which was dramatically induced by bacterial infection with a more than 16-fold expression change ($\log_2$FC > 4) (Fig. 3B). *In vitro*, it has been demonstrated that LPS has the capacity to increase *apoD* expression by influencing transcription factors, such as nuclear factor-kappa B, activation protein 1, acute phase responsive element and hepatocyte nuclear factor-1α [43]. In our study, the expression levels of *apoEa* and *apoEb* in the liver were significantly repressed by infection, which is consistent with previous observations in mammals [44,45]. Previous reports revealed that the repression of *apoEa* and *apoEb* might be due to LPS, which could directly inhibit *apoE* promoter activity via mitogen-activated protein kinase signalling pathways and certain transcription factors, such as activation protein 1 and nuclear factor-kappa B [45]. In addition, *apoM* exhibited a decreased expression pattern in response to LPS in the liver at 24 h after infection (Fig. 3C), which is consistent with the findings in olive flounder (*Paralichthys olivaceus*) [46]. The repression of *apoM* has also been reported in mice and is caused by tumour necrosis factor or interleukin-1 cytokines [47]. Teleost-specific *apo-14kDa* of spotted sea bass was significantly induced in the liver in response to bacterial infection (Fig. 3C), which is consistent with its expression profile in carp [13], European sea bass (*Dicentrarchus labrax*) [48], and Atlantic salmon [16]. As the homologue of mammalian *apoA2*, *apo-14kDa* may exhibit antimicrobial activity against bacteria [17].

In the intestine, up-regulation of 13 tested *apo* genes was observed, while 6 *apo* genes were significantly down-regulated after bacterial infection. Although the expression varied among the genes, the expression level of 14 genes changed more than 4-fold ($\log_2$FC > 2), implying their important roles in the immune response in the intestine. Significant up-regulation of *apoA1* and *apoA4a.1* was also detected in the intestine, while their expression levels peaked at 24 h post-infection compared with the peak at 12 h post-infection in the liver (Fig. 3D). It was widely reported that bacterial infection begins in intestine and bacterial products, including LPS, are then brought to liver via portal venous blood [49–51]. However, not all immune-related genes are firstly activated in intestine. For example, the expression levels of *Major histocompatibility complex class II A* (*MHC class II A)* genes, an important component of primary immune defense, were significantly up-regulated and reached the peak in liver at 24 h post *Vibrio anguillarum* injection in half-smooth tongue sole (*Cynoglossus semilaevis*) compared with 48 h post injection in intestine [52]. The expression of both *apoC1* and *apoC2* were significantly induced in the liver, while their expression was repressed in the intestine (Fig. 3A, D). The expression of *apoC1* and *apoC2* in teleosts exhibited drastic tissue differences after infection, implying distinct immune functions between the liver and intestine. Previous studies have mainly focused on *apoC1* and found that *apoC1* is induced or repressed in different tissues and teleosts after infection. In turbot (*Scophthalmus maximus*), the expression of *apoC1* in the spleen was significantly up-regulated, with a 4-fold change after *Aeromonas salmonicida* infection [53]. Similar observations have been reported by Hwang et al. [46], who demonstrated that *apoC1* is up-regulated in the kidneys of haemorrhagic septicaemia virus-infected olive flounder. In contrast, *apoC1* expression is repressed in the liver of ayu (*Plecoglossus altivelis*) after *Listonella anguillarum* infection [54]. Furthermore, an *in vitro* study indicates that *ApoC1* contains structural elements in both its N-terminal and C-terminal helix to bind LPS, to modulate the behaviour of LPS and to enhance the Toll-like receptors 4-dependent proinflammatory response towards LPS via an LPS binding protein-like mechanism [55]. Notably, *apoO* was found to be dramatically up-regulated in both the liver and intestine after bacterial infection (Fig. 3C, F). Although no experimental evidence is available to illustrate the specific roles of *apoO* in the defence response after infection, the dramatic induction of expression suggested that it could be important in the defence response against *V. harveyi* infection in spotted sea bass. In addition, *apoL*, *apoF* and *apoOL* were also significantly influenced by bacterial infection, particularly *apoF,* which exhibited tissue-specific expression differences after infection, suggesting that *apoF* may be involved in distinct biological functions in the liver and intestine in spotted sea bass. However, the related immune functions of *apoL*, *apoF* and *apoOL* remain unknown, and the detailed mechanism requires further research.

In summary, a complete set of 23 *apo* genes was identified and annotated in spotted sea bass. Using phylogenetic and homology analysis, tandem arrangements and duplication events among *apo* genes in spotted sea bass were discovered. Furthermore, a total of 20 *apo* genes were differentially expressed in the liver and intestine of spotted sea bass after *V. harveyi* infection, suggesting their potential roles in the immune response. Our study provides a valuable foundation for future studies aimed at uncovering the specific roles of each apolipoprotein during bacterial infections in teleosts.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fsi.2019.06.005.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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