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# Genome wide identification of scavenger receptors class A in common carp (*Cyprinus carpio*) and their expression following *Aeromonas hydrophila* infection

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ABSTRACT

Scavenger receptors class A (SCARAs) is a subgroup of diverse families of pattern recognition receptors that bind a range of ligands, and play important roles in innate immune processes through pathogens detection, adhesion, endocytosis, and phagocytosis. However, most studies of SCARAs have focused on mammals, and much less is known of SCARAs in fish species. In this study, we identified 7 SCARAs across the common carp genome, which were classified into four subclasses according to comparative genomic analysis including sequence similarities analysis, gene structure and functional domain prediction. Further phylogenetic and syntenic analysis supported their annotation and orthologies. Through examining gene copy number of SCARA genes across several vertebrates, SCARA2, SCARA3 and SCARA4 were found have undergone gene duplication. The expression patterns of SCARAs in common carp were examined during early developmental stages, in healthy tissues, and after *Aeromonas hydrophila* infection. Most SCARA genes were ubiquitously expressed during common carp early developmental stages, and presented diverse patterns in various healthy tissues, with relatively high expression levels in spleen, liver, intestine, gill and brain, indicating their critical roles likely in maintaining homeostasis and host immune response activities. After *A. hydrophila* infection, most SCARA genes were up-regulated at 4 h post infection in mucosal tissue intestine, while generally up-regulated at 12 h post infection in spleen, suggesting a tissue-specific pattern of regulation. Taken together, all these results suggested that SCARA genes played important roles in host immune response to *A. hydrophila* infection in common carp, and provided important genomic resources for future studies on fish disease management.



## 1. Introduction

Scavenger receptors (SRs) are a structurally diverse group of pattern recognition receptors (PRRs) with a striking range of functions, including lipid transport, foreign ligands recognition and clearance of effete host cells or pathogens during the early stages of infection [1]. SR was initially discovered in 1970s [2]. Later on, a variety of SRs have been reported, and categorized into nine classes (A, B, C, D, E, F, G, H and I) according to their structural characteristics and functional properties [3]. Scavenger receptors class A (SCARAs) are significant PRRs, and are involved in pathogen recognition, adhesion, endocytosis, phagocytosis, as well as in a range of macrophage-associated pathophysiological processes, such as diabetes, and myocardial infarction [4–8]. SCARAs consist of 5 members, including macrophage scavenger receptor 1 (SCARA1/SR-A1/MASR1), macrophage receptor with collagenous structure (SCARA2/MARCO), cellular stress response protein (SCARA3/CSR1), scavenger receptor with C-type lectin (SCARA4/CLP1/COLEC12), and scavenger receptor class A member 5 (SCARA5/TESR). All five SCARA members have similar structures, containing a collagenous domain, and might also having a type A scavenger receptor cysteine-rich (SRCR) domain or a C-type lectin (CLEC) domain [3], but the lengths of their collagenous domains at

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the C-terminal end vary considerably [9].

The roles of SCARAs in immunity have been extensively studied. SCARA1, for instance, has been showed that it can bind to lipopolysaccharide (LPS) which is an integral component of Gram-negative cell walls [10], and lipoteichoic acid (LTA) expressed in Gram-positive bacteria [11]. Therefore, SCARA1 can mediate the non-opsonic uptake of bacteria such as *Staphylococcus aureus* [12], *Neisseria meningitides* [13], and *Listeria monocytogenes* [14]. SCARA2, structurally similar to SCARA1, also can recognize LPS, LTA, and bind to bacteria [15]. Using knockout mice (*Mus musculus*) showed another role of SCARAs in immunity that it could eliminate invading microbes from the host [16]. Besides pathogen recognition and removal, SCARAs are able to interact with other PRRs like Toll-Like Receptors (TLRs) and therefore influence signaling pathway. Amiel et al. [17] found that SCARA1 interacted with TLR4 to promote the phagocytosis of the Gram-negative bacterium *E. coli*, while SCARA1 was cooperated with TLR2 in the phagocytosis of the Gram-positive bacterium *S. aureus*.

Knowledge on the SCARA family including its possible roles in host immunity, mainly came from investigations in the mammalian system, however, only a few studies had been reported from the ancient vertebrate, teleost. For instance, knockdown of SCARA4 (COLEC12) in zebrafish (*Danio rerio*) showed that it was implicated in vasculogenesis [18]. Teleost SCARA5 was first cloned and functional characterized from spotted green pufferfish, *Tetraodon nigroviridis*, and subcellular localization analysis showed that SCARA5 was a cell membrane receptor with homotrimer forms involved in the recognition and internalization of LPS from surface membranes into lysosomes [19]. SCARA2, SCARA3 and SCARA5 were identified in large yellow croaker (*Pseudosciaena crocea*) and all of them were up-regulated in spleen after *Vibrio alginolyticus* injection [20]. In rainbow trout (*Oncorhynchus mykiss*), SCARA ligands LTA and formaldehyde treated bovine serum albumin (fBSA) modify the uptake of mLDLs in kidney [21]. Also the function and expression of SCARAs were investigated in the rainbow trout cell lines, RTgutGC and RTgill-W1 [22], as well as in nonspecific cytotoxic cells (NCC) of catfish (*Ictalurus punctatus*), tilapia (*Oreochromis niloticus*) and cod (*Gadus morhua*) [23–26].

Common carp, *Cyprinus carpio*, one of the most economically important aquaculture fish species, is cultured in over 100 countries worldwide and accounts for up to 10% of global annual freshwater aquaculture production [27,28]. The intensive rearing of common carp in aquaculture has led to a high susceptibility to various disease agents, such as *Aeromonas hydrophila*, which is one of the most common bacterial pathogens in freshwater habitats throughout the world [29]. For the purpose of generating effective strategy to better manage fish disease, it is important to understand the immune-related functional genes as well as their expression during bacterial infection. In this study, utilizing all available genomic resources, we reported the genome-wide identification of SCARA genes in common carp. Their sequence structures and functional domains were analyzed. Further phylogenetic and syntenic analysis confirmed the annotation. Moreover, the expression patterns of SCARA genes during common carp early developmental stages, in healthy tissues, and after *A. hydrophila* infection were examined. Our systematic study of SCARA genes on the common carp provided fundamental genomic resources for better understanding the critical roles of SCARAs on the primary innate immune processes in teleost.

## 2. Materials and methods

### 2.1. Ethics statement

All sampling procedures involving the handling and treatment during this study were approved by the Animal Care and Use committee of the Centre for Applied Aquatic Genomics at the Chinese Academy of Fishery Sciences prior to initiation. The fish were euthanized by using MS-222 before sampling, and all efforts were made to minimize suffering.

### 2.2. Bacterial challenge and sample collection

6-month old common carp (20–30 cm in length, 300–400 g in weight) were collected from common carp farms located in Zhengzhou, Henan, China, and immediately transferred to the laboratory. After two weeks of acclimatizing at 25 °C in tanks with 100 L filtered fresh water, fish were randomly divided into control group and treatment group. Treated group was intraperitoneally injected with 0.1 ml of *A. hydrophila*, cultured in LB medium ($1 \times 10^8$ CFU/ml), while control group was injected with same volume of sterilized LB broth. Spleen and intestine were collected at 4 h, 12 h, and 24 h post challenge. At each time point, 15 fish from each group were randomly selected and divided into 3 replicates (5 fish per replicate). Tissues were immediately submerged into 10 ml RNAlater™ (Ambion, USA), following the manufacturer's protocol, and stored at −80 °C until RNA extraction.

### 2.3. SCARAs genes identification and sequence analysis

All available SCARAs genes from zebrafish and human were obtained from Ensemble (http://asia.ensembl.org) and NCBI (http://www.ncbi.nlm.nih.gov/) databases, and used as the query sequences to search against all available common carp genomic resource by BLAST tools, with an *E*-value cutoff of 1e-5. Then reciprocal BLAST searches were conducted by using the candidate common carp SCARA genes as queries to verify the veracity of candidate genes. Additionally, the coding sequences were confirmed by BLAST searches against NCBI non-redundant protein sequence database (nr). The exon-intron structure analysis was conducted using the Fancy Gene 1.4 online analysis tool (http://bio.ieo.eu/fancygene/). The simple modular architecture research tool (SMART) was used to predict the conserved domains based on sequence homology and further confirmed by conserved domain prediction from BLAST.

### 2.4. Phylogenetic analysis

Phylogenetic analysis was performed using SCARA proteins from common carp and other organisms including human (*Homo sapiens*), mouse, chicken (*Gallus gallus*), lizard (*Anolis carolinensis*), frog *(Xenopus tropicalis)*, medaka (*Oryzias latipes*), fugu (*Takifugu rubripes*), stickleback (*Gasterosteus aculeatus*), coelacanth (*Latimeria chalumnae*), tilapia, cod and zebrafish. Multiple protein sequences were aligned by ClustalW with default parameters. We performed maximum likelihood analysis in MEGA6 [30] with bootstrap test of 1000 replicates. The best-fit model was the JTT + I + G model which uses a Jones-Taylor-Thornton (JTT) matrix and incorporates a proportion of invariant sites (+I) and the gamma distribution for modeling rate heterogeneity (+G). The maximum likelihood tree was constructed using MEGA6 with Subtree-Pruning-Regrafting – Extensive (SPR level 5) as the LM Heuristic Methods.

### 2.5. Expression of SCARA genes during common carp early developmental stages and in healthy tissues

Pooled samples of 10 egg mass/fries from eight early developmental stages post-fertilization (0 h, 12 h, 24 h, 36 h, 48 h, 3 days, 5 days, and 7 days) and nine healthy tissues from 3 adult common carps (brain, heart, spleen, liver, kidney, intestines, gill, muscle and