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Molecular characterization of nineteen cytokine receptor family B (CRFB) members, CRFB1, CRFB2, CRFB4-17, with three CRFB9 and two CRFB14 in a cyprinid fish, the blunt snout bream *Megalobrama amblycephala*

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ABSTRACT

The class II cytokine receptor family members are receptors of class 2 helical cytokines in mammals, and are named cytokine receptor family B (CRFB) in fish. In zebrafish, sixteen members, including CRFB1, CRFB2 and CRFB4-17 were reported. With the availability of genome sequence, a total of nineteen CRFBs was identified in the blunt snout bream (*Megalobrama amblycephala*), including CRFB1, CRFB2, CRFB4-17 with the presence of three CRFB9 isoforms, and two CRFB14 isoforms. These CRFB molecules contain well conserved features, such as fibronectin type III (FNIII) domain, transmembrane and intracellular domains as other class II cytokine receptors, and are phylogenetically grouped into thirteen clades with their homologues from other species of fish. The CRFB genes were constitutively expressed in organs/tissues examined in the fish. The finding of more CRFB members in the bream may provide clues to understand possible receptor-ligand interaction and their diversity from an evolutionary point of view.

1. Introduction

Cytokines, a group of small proteins (~5 – 20 kDa) produced mainly by immune cells, can regulate a variety of biological processes such as immune response, inflammation, and embryonic development through their transmembrane receptors (Dinarello, 2007; Oppenheim, 2001). In mammals, cytokines are separated into β-trefoil cytokines, β-jellyroll cytokines, cysteine knot cytokines, and two classes of helical cytokines, i.e. class 1 and class 2 helical cytokines because of their similar four-alpha-helix bundle (Dembic, 2015; Oppenheim, 2018; Zou and Secombes, 2016). Class 2 helical cytokines contain the IL-10 family members, i.e. IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, and types I, II, III (also as IL-28A, IL-28B, IL-29) and IV interferons (IFNs), and they use the class II cytokine receptor family (CRF2, also known as class II helical cytokines receptor, HCRII) members, which lack the complete tryptophan-serine-X tryptophan-serine (WSYWS) motif, being thus different from the motif-containing CRF1 members (Chen et al., 2022b; Huising et al., 2006; Krause and Pestka, 2005; Langer et al., 2004; Pestka et al., 2004; Sabat, 2010). The intracellular domain of helical cytokines is required to bind to tyrosine kinases for initiating corresponding signalling pathways, such as JAK/STAT pathways (Javed et al., 2010; Krause and Pestka, 2005; Langer et al., 2004).

In mammals, CRF2 may be subdivided into long-chain and short-chain receptor subunits according to the length of intracellular region (Langer et al., 2004). The long-chain receptors are IFNAR2, IFNGR1, IL-10R1, IL-20R1, IL-22RA1 and IFNLR1, and they usually have a high

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affinity for their ligands; short chain receptors include IFNAR1, IFNGR2, IL-10R2 and IL-20R2 (Huising et al., 2006; Krause and Pestka, 2005; Langer et al., 2004; Trivella et al., 2010). In addition to the above transmembrane members, there is a secretory receptor named IL-22 binding protein (IL-22BP) or IL-22RA2 (Huo et al., 2019a; Xu et al., 2001). Tissue factor (TF) participates in innate immunity by binding to Factor VII/VIIa (FVII/FVIIa) and activating multiple signalling pathways in the coagulation cascade to prevent blood loss during injury and to limit the spread of infection from the wound to the body (Golino, 2002; Lutfalla et al., 2003).

CRF2 members are characterized with three structural regions, an extracellular domain, a transmembrane domain, and an intracellular domain (Krause and Pestka, 2005; Mogensen et al., 1999; Monte et al., 2015). The extracellular region of most CRF2 members possesses two fibronectin type-III (FNIII) domains, a structural motif in the immunoglobulin fold superfamily, contained in the D200 domain of IFN long-chain receptors (Langer et al., 2004; Lutfalla et al., 2003; Monte et al., 2015). However, the low-affinity receptor chain IFNAR1 contains four FNIII subdomains (SD), SD1−SD4, depending on their proximity to the membrane, which are most likely derived from gene duplication (Langer et al., 2004; Piehler et al., 2012; Uzé et al., 1990; Wang et al., 2022). It is hypothesized that all class II ligand/receptor systems have a common ancestor in terms of genetic and structural data (Lutfalla et al., 2003; Stein et al., 2007). Homologues of CRF2 family are also present in fish, but one-to-one homology cannot be directly found in homologues of CRF2 members in fish with mammalian CRF2 family members except for tissue factor (TF) (Krause and Pestka, 2005; Langer et al., 2004; Levraud et al., 2007; Lutfalla et al., 2003; Monte et al., 2015; Stein et al., 2007).

In fish, CRF2 was named cytokine receptor family B (CRFB), and a total of eleven genes, CRFB1 to CRFB11, were first discovered in pufferfish (*Tetraodon nigroviridis*) (Lutfalla et al., 2003). Later, sixteen genes encoding CRFB members were identified in zebrafish, namely CRFB1, CRFB2 and CRFB4-11 (Levraud et al., 2007), CRFB12-16 (Stein et al., 2007), CRFB17 (Aggad et al., 2010). CRFB9 is highly similar to IL-22BP (IL-22RA2) without transmembrane domain (Aggad et al., 2010; Huo et al., 2019a). CRFB10 and CRFB11 are two homologous copies of TF (Lutfalla et al., 2003; Sprague et al., 2006; Stein et al., 2007). CRFB1, CRFB2 and CRFB5 are the receptor complex for type I IFNs (Aggad et al., 2009; Chen et al., 2022a; 2022b; Gan et al., 2020; Laghari et al., 2018; Levraud et al., 2007), and type II IFNs use CRFB6 (IFNGR2), CRFB13 (IFNGR1-2) and CRFB17 (IFNGR1-1) (Aggad et al., 2010; Chen et al., 2013; Li et al., 2019; Zhu et al., 2022), type IV IFN uses CRFB4 (IL-10R2) and CRFB12 (IFN-υR1) (Chen et al., 2022b). In addition, homologous relationships in CRFB7 (IL-10R1), CRFB8 (IL-20R1), CRFB14 (IFNLR1, IL-22RA1), CRFB16 (IL-20R2) between fish and mammals have also been discovered, but no related homologous genes of CRFB15 have been reported (Aggad et al., 2010; Chen et al., 2022b; Huo et al., 2021; Levraud et al., 2007; Lutfalla et al., 2003; Stein et al., 2007). This may imply vast differentiation of class II cytokines and their receptors in fish, making homology ambiguous (Aggad et al., 2010; Gan et al., 2018; Lutfalla et al., 2003; Monte et al., 2015; Stein et al., 2007).

The blunt snout bream (*Megalobrama amblycephala*), which is a cyprinid fish, has an economic importance in aquaculture (FAO, 2018) probably because of its eco-friendly and resource-saving characteristics (Chen, 1998; Liu et al., 2017, 2021). Up to now, the genome of this fish has been sequenced by two independent groups in China, and the presence of all CRFB genes reported in zebrafish has been identified in the genome (Liu et al., 2017, 2021; Ren et al., 2019). However, any report on CRFBs in the same fish is not available in literature. In the current study, the entire open reading frames (ORF) of sixteen CRFB genes were cloned in blunt snout bream, including CRFB1, CRFB2, CRFB4, CRFB5, CRFB6, CRFB7, CRFB8, CRFB9 (including three transcriptional isoforms, CRFB9a, CRFB9b, and CRFB9c), CRFB10, CRFB11, CRFB12, CRFB13, CRFB14 (including two transcriptional isoforms, CRFB14a and CRFB14b), CRFB15, CRFB16 and CRFB17. The sequence characteristics and phylogeny were analyzed bioinformatically, and quantitative real-time PCR was carried out to examine the constitutive expression of CRFB genes. The findings of nineteen CRFBs in blunt snout bream will provide potential molecular basis to explore receptor and ligand interaction for class II cytokines and to further understand their functions in immune system.

2. Materials and methods

2.1. Fish

A total of forty blunt snout bream (*Megalobrama amblycephala*), about 5 g each, was obtained from a fish farm in Chongqing city, China. These fish were maintained in aerated freshwater aquarium at 25 °C and fed with commercial feed for at least two-week acclimatization before further experiments.

All animal experiments were performed following the Guide for the Care and Use of Laboratory Animals of the Chinese Academy of Sciences and were approved by the Institute of Hydrobiology.

2.2. RNA extraction and gene cloning

Total RNA using TRIzol® reagent (Invitrogen, USA) was extracted from mixed samples of organs/tissues in one healthy blunt snout bream, and was used to synthesize the first-strand cDNA template by using RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) as described by the manufacturers.

The partial sequences of CRFB1, CRFB2, CRFB4, CRFB5, CRFB6, CRFB7, CRFB8, CRFB9a, CRFB9b, CRFB9c, CRFB10, CRFB11, CRFB12, CRFB13, CRFB14a, CRFB14b, CRFB15, CRFB16 and CRFB17 were obtained from the genome sequence of blunt snout bream (genome assembly no. ASM1881202v1, https://www.ncbi.nlm.nih.gov/assembly/GCF_018812025.1/) (Liu et al., 2021), and were compared with homologous sequences in other species of fish using the Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information website (NCBI, http://blast.ncbi.nlm.nih.gov/Blast.cgi).

The synthesized cDNA was used to clone the ORFs of CRFBs with specific primers (listed in Supplementary Table 1; Supplementary Fig. 1), which were designed using primer 5.0. Phanta® Super Fidelity DNA Polymerase (Vazyme, China) was used for PCR amplification as the following programme: one cycle of 95 °C for 3 min, 34 cycles of 95 °C for 15 s, 55 °C for 15 s and 72 °C for 2 min, finally one cycle of 72 °C for 5 min. All PCR products were checked by agarose gel electrophoresis, and cloned into the pMD18-T vector (TaKaRa, Japan) for sequencing by Sangon Biotech Co., Ltd (Shanghai, China).

2.3. Bioinformatics analysis

The protein sequences, signal peptide sequences, N-glycosylation sites and transmembrane regions were predicted using translate tool ExPASy server (http://web.expasy.org/translate/), SignalP4.1 program (http://www.cbs.dtu.dk/services/SignalP/), ScanProsite tool (https://prosite.Expasy.org/scanprosite/) and TMHMM Server (version 2.0) (http://www.cbs.dtu.dk/services/TMHMM/). Multiple alignment of protein sequences was performed through the CLUSTAL W program (version 1.8) and rendered in GeneDoc software (version 2.7.0). Aligned amino acid sequences were used to conduct phylogenetic analysis using the neighbor-joining (NJ) method with bootstrap analysis of 10,000 times in MEGA X software. All nucleic acid and amino acid sequences used in the analysis were from NCBI, and were listed in Supplementary Tables 2 and 3.

2.4. Expression analysis of CRFB genes in blunt snout bream

Various organs/tissues including trunk kidney, head kidney, spleen, liver, heart, muscle, gill, intestine and brain in four healthy blunt snout

breams were separately collected for analyzing the constitutive expression of CRFBs.

Total RNA from different organs/tissues was separately extracted by using TRIzol® reagent (Invitrogen, USA), which was used to synthesize the first-strand cDNA template using RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) following the manufacturer's instruction.

Quantitative real-time PCR (qRT-PCR) was performed withy Bio-Rad CFX96 Real-Time System with 10 μl iQ™ SYBR Green mix (Bio-Rad, Singapore), 1 μl forward primer, 1 μl reverse primer (Supplementary Table 1; Supplementary Fig. 1), 1 μl cDNA template and 7 μl sterile water under following cycling parameters: 3 min at 95 °C for initial denaturation, followed by 40 cycles at 95 °C for 5 s, 60 °C for 20 s, 72 °C for 20 s. All samples were tested in triplicates. All the products were connected to the pMD18-T vector and transformed into the *Escherichia coli* Top 10. The positive clones were sequenced by Sangon Biotech. Plasmid Mini Kit (Omega) was used to extract the sequenced bacterial solution from the positive clone. The plasmid was diluted in a 10-fold gradient and measured by spectrophotometer (Nano Drop 2000; Thermo Scientific) to create standard curves ranging from 10^{-6} to 10^{-2}. That relative change was calculated by using the method of $2^{-\Delta\Delta ct}$, and the fold change relative to the expression level in corresponding control group was normalized against *ef1a* gene.

2.5. Statistical analyses

All data were analyzed in GraphPad Prism 6.0 and SPSS 23.0 software based on three repeated experiments. The difference in relative gene expression was analyzed using independent samples *t*-test. Error bars were presented as the mean ± standard error (SE), with * indicating $P < 0.05$, $^{**}P < 0.01$.

3. Results

3.1. CRFB genes in blunt snout bream

Sixteen CRFBs, CRFB1, CRFB2, CRFB4-17, were found in the blunt snout bream, with the presence of three isoforms of CRFB9, CRFB9a, CRFB9b, CRFB9c, and two isoforms of CRFB14, CRFB14a, CRFB14b (GenBank accession numbers listed in Supplementary Table 3). The amino acid identity (%) between all CRFB genes in blunt snout bream was presented in Supplementary Table 4. Different members of CRFBs showed a relatively low level of identity, and CRFB9a, CRFB9b and CRFB9c had high-level identity of greater than 90%, and CRFB14a and CRFB14b had an identity of 99.1% (Supplementary Table 4).

Furthermore, the first 18, 19, 20, 21, 22, 21, 19, 28, 26, 29, 22, 22, 18, 27, 10, and 20 aa were predicted in CRFB1, CRFB2, CRFB4, CRFB5, CRFB6, CRFB7, CRFB8, CRFB9a, CRFB10, CRFB11, CRFB12, CRFB13, CRFB14a, CRFB15, CRFB16 and CRFB17 as signal peptides, respectively. Signaling peptide was not forecasted in CRFB9b, CRFB9c and CRFB14b. Chromosomal localization of CRFB members are listed in Supplementary Table 3.

3.2. Sequence features and phylogenetic analysis of CRFBs in blunt snout bream

The alignment of these CRFB amino acid sequences in blunt snout bream showed common and well conserved features as other class II cytokine receptors, including the fibronectin type III (FNIII) domain, signal peptide at the N terminus, a transmembrane domain. Two putative STAT activating tyrosine (Y) motifs and one putative Tyk2 association domain were found in the intracellular region of CRFB1, CRFB2 and CRFB5 in blunt snout bream as in other species of fish (Supplementary Fig. 2). Gene synteny analysis revealed that CRFB1, CRFB2, CRFB4 and CRFB5 are adjacent to each other on the same chromosome (Supplementary Fig. 3A), and that CRFB7, CRFB14, CRFB15 and CRFB16 loci in blunt snout bream are highly conserved with zebrafish and other vertebrates (Supplementary Figs. 3B, F, G, H). Similarly, CRFB13 and CRFB17, and CRFB10 and CRFB11 in blunt snout bream and zebrafish are duplicated genes of mammalian IFNGR1 and TF, respectively (Supplementary Figs. 3C and D).

In general, these CRFBs from blunt snout bream were clustered into thirteen major branches in the phylogenetic tree. CRFB1 and CRFB2 were clustered in the same branch with IFNAR2 in mammals, and were closely related with homologues in cyprinids. CRFB5 and IFNAR1 were all in a same clade, which all have short intracellular chain (Fig. 1). CRFB4 was clustered with IL-10RB from other species, showing closest relationship with grass carp and common carp CRFB4. CRFB6 was clustered together with IFNGR2. CRFB13 and CRFB17 were grouped together with IFNGR1, and were closely related with CRFB13 and CRFB17 in grass carp and zebrafish. CRFB7, CRFB8, CRFB9, CRFB14, and CRFB16 were clustered with IL-10RA, IL-20RA, IL-22BP, IL-22RA1 and IL-20RB, respectively. CRFB10 and CRFB11 were grouped within other bony fish and other vertebrate TF cluster supported by a high bootstrap value (Fig. 1).

3.3. Constitutive expression of CRFB genes in vivo

The CRFBs had various expression levels in all tested organs/tissues, including liver, spleen, head kidney, trunk kidney, intestine, gill, muscle, heart and brain from the healthy blunt snout bream (Fig. 2A-Q). CRFB1, CRFB2, CRFB4, CRFB5, CRFB6, CRFB7, CRFB11 and CRFB13 had highest expression level in spleen. On the contrary, the lowest mRNA level of these CRFBs was found in muscle except CRFB11 (Fig. 2A, B, C, D, E, F, G, H, I, K, M). Furthermore, CRFB8, CRFB14a, CRFB14b and CRFB17 had relatively high expression level in intestine (Fig. 2G, N, O, Q). CRFB9a and CRFB9c were mainly expressed in brain and trunk kidney, respectively. In addition, CRFB10, CRFB12 and CRFB15 exhibited the highest expression level in gill. On the other hand, CRFB10, CRFB14a and CRFB17 had relatively low expression in head kidney, and the expression of CRFB11, CRFB12 and CRFB15 was low in heart, and CRFB14b in liver (Fig. 2J, K, L, N, O, P, Q).

4. Discussion

In this study, nineteen CRFB members were identified in blunt snout bream, which were named as CRFB1, CRFB2, CRFB4, CRFB5, CRFB6, CRFB7, CRFB8, CRFB9a/CRFB9b/CRFB9c, CRFB10, CRFB11, CRFB12, CRFB13, CRFB14a/CRFB14b, CRFB15, CRFB16 and CRFB17 based on conserved motifs, sequence characterization and phylogenetic analysis. The finding of nineteen CRFBs in the present study may represent the finding of largest number of CRFB members in a single species of fish so far, which should provide basis for further research on ligand-receptor interactions.

With the development of comparative genomics, a total of sixteen CRFB genes was initially identified through genome analysis in pufferfish and zebrafish (Aggad et al., 2009, 2010; Lutfalla et al., 2003; Stein et al., 2007), and the possible functions of these CRFB genes have been investigated over the last two decades (Chang et al., 2013; Chen et al., 2022a, 2022b; Huo et al., 2021; Huo et al., 2019b; Monte et al., 2015; Quiniou et al., 2022; Sun et al., 2014; Wang et al., 2021; Wang et al., 2022; Zhu et al., 2022; Zou and Secombes, 2016).

As type I IFN receptor complex, CRFB1/CRFB2 and CRFB5 are homologues of mammalian IFNAR2 and IFNAR1, respectively (Aggad et al., 2009; Gan et al., 2020; Hamming et al., 2011; Levraud et al., 2007; Li et al., 2020). Knockdown, crosslink and co-transfection experiments have shown that CRFB1 and CRFB5 interacts with group I IFNs, while group II IFNs bind with CRFB2 and CRFB5 for functioning (Aggad et al., 2009; Chen et al., 2015; Laghari et al., 2018). Homoplastically, in blunt snout bream and other bony fish, CRFB5 differs from mammalian IFNAR1, which contains only two FNIII domains rather than four FNIII domains in mammals and birds (Gan et al., 2018, 2020; Stein et al.,

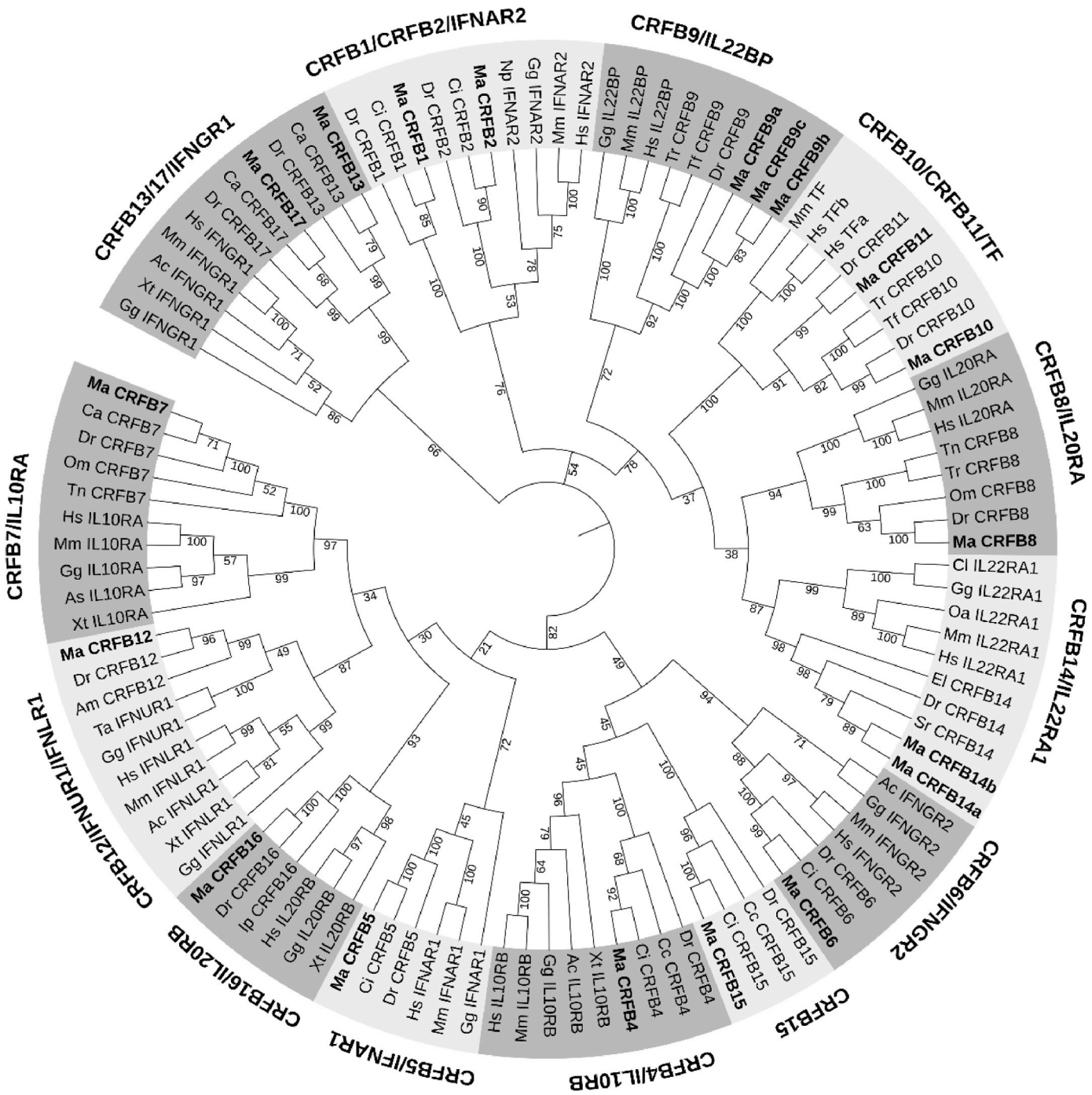

Fig. 1. Phylogenetic analysis of nineteen CRFB members, CRFB1, CRFB2, CRFB4, CRFB5, CRFB6, CRFB7, CRFB8, CRFB9a, CRFB9b, CRFB9c, CRFB10, CRFB11, CRFB12, CRFB13, CRFB14a, CRFB14b, CRFB15, CRFB16 and CRFB17 in blunt snout bream with their counterparts in other species. The predicted amino acid sequences of CRFBs in blunt snout bream were used in the phylogenetic tree construction using neighbor-joining method in Mega X program with 10,000 replicates of bootstrap analysis. The CRFBs used to construct the phylogenetic tree are listed in Supplementary Table 2.

2007; Stosik et al., 2021). In a recent research, the crystal structure of IFNa in grass carp (*Ctenopharyngodon idella*), which has two cysteines and should be clarified as group I IFN, was demonstrated to bind with CRFB1, CRFB2 and CRFB5 (Wang et al., 2022), implying possibly that IFNa may interact with CRFB1 and CRFB5, or CRFB2 and CRFB5.

IFN-γ and its receptors in teleost fish are replicated due to the teleost-specific whole-genome duplication (TGD) (Gan et al., 2020; Pang et al., 2023; Secombes and Zou, 2017). In zebrafish, IFN-γ binds to CRFB6, CRFB13 and CRFB17, and IFN-γrel binds to CRFB17 (Aggad et al., 2010). However, studies on crystal structure of IFN-γrel in grass carp showed that it does not bind to CRFB6, but binds to CRFB17 and CRFB13 by examining STAT1 phosphorylation (Zhu et al., 2022). The conclusion of co-transfection experiment in mandarin fish (*Siniperca chutatsi*) indicated that IFN-γ combines with CRFB6 and CRFB13, and IFN-γrel combines with CRFB17 (Li et al., 2019). The high expression level of CRFB13 in blunt snout bream, as reported in other fish, such as mandarin fish (Li et al., 2019), and also the significantly induced expression level of CRFB17 in the bream may imply their involvement in antiviral immunity in fish. But, the relationship between the two type II IFNs and corresponding receptors CRFB6, CRFB13 and CRFB17 needs further

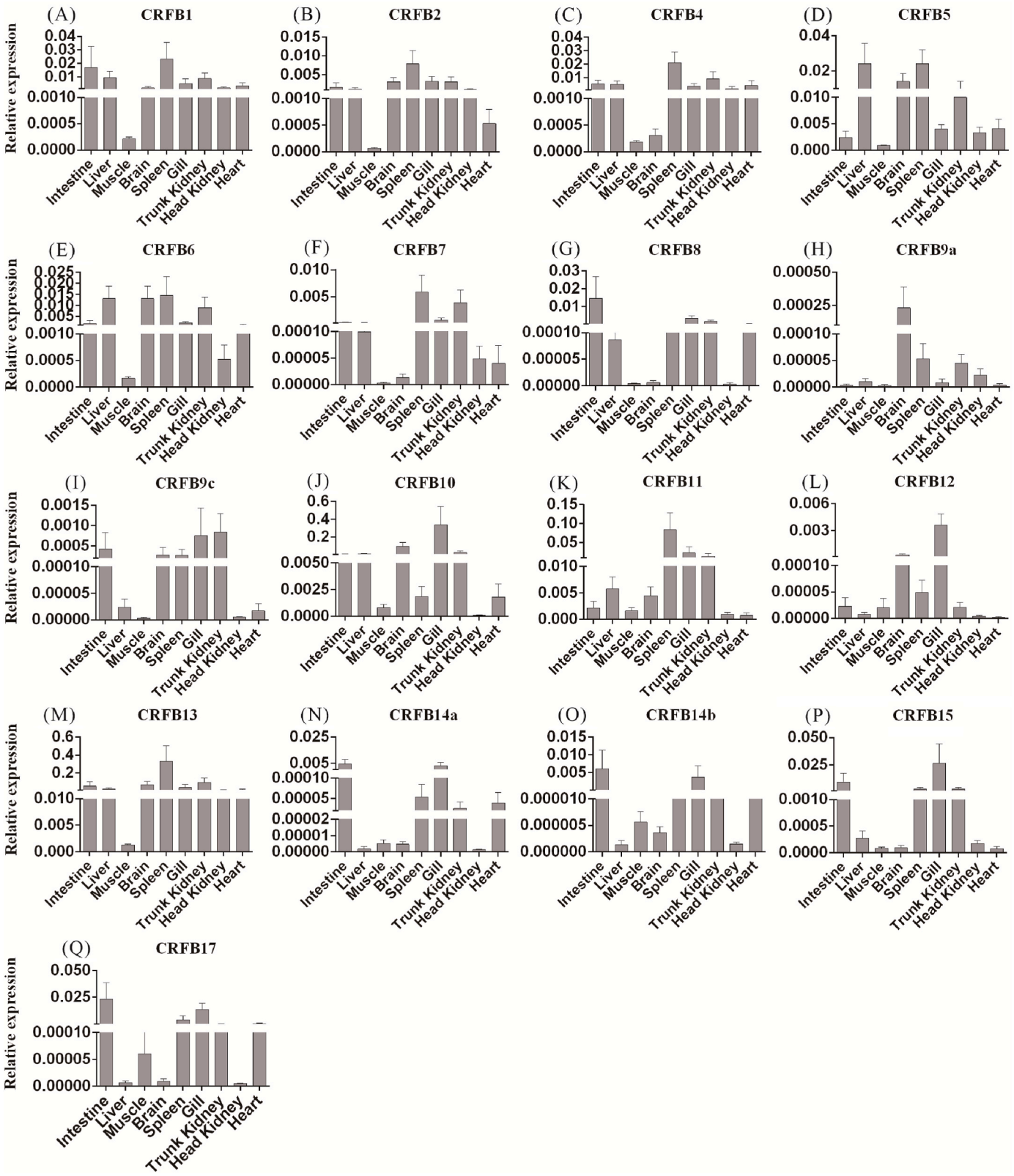

Fig. 2. The expression of CRFBs in healthy blunt snout bream (n = 4). Gene expression was measured by qRT-PCR and the level was normalized against the level of *ef1a*. The expression of CRFB9b was not detected. Data were expressed as mean ± SE.

exploration.

IL-10R2/CRFB4 is a receptor subunit involved in the activation of some members of class II cytokines such as IL-10, IL-22, IL-26 and type III IFN, and is constitutively expressed in mammals and fish (Chen et al., 2022b; Huo et al., 2019b; Monte et al., 2015; Trivella et al., 2010; Wei et al., 2014; Wolk et al., 2002; Xu et al., 2021). Recently, a formerly uncharacterized IFN, type IV IFN, named as IFN-υ was identified in zebrafish, clawed frog, and in black carp (*Mylopharyngodon piceus*)

(Chen et al., 2022b; 2023; Su, 2022), and IL-10R2/CRFB4 and CRFB12 are the receptor complex of IFN-υ, which differs from the receptors of type I, II and III IFNs. It is proved that IL-10R2/CRFB4 is shared by IFN-υ and type III IFNs as a receptor complex chain, although type III IFNs are not reported in teleost fish (Chen et al., 2022b; Redmond et al., 2019). Moreover, CRFB7/IL-10R1, another IL-10 transmembrane receptor, is reported to have high expression level in spleen, kidney, intestine and gills of zebrafish, mandarin fish, goldfish and grass carp (Grayfer and Belosevic, 2012; Huo et al., 2019b; Wei et al., 2014), as reported in blunt snout bream in the present study. The two receptors for IL-10 in mandarin fish all responded to ISKNV infection (Huo et al., 2019b), with IL-10R2 expression being significantly higher than IL-10R1 in mandarin fish, as observed in blunt snout bream (Huo et al., 2019b). However, the increased expression of IL-10R2 may imply its role in functional signalling of other cytokines in host defense (Chen et al., 2022b; Huo et al., 2019b; Monte et al., 2015).

The CRFB10 and CRFB11 in *T. nigroviridis* are homologous to mammalian transcription factors with similar intron/exon structures (Lutfalla et al., 2003). In blunt snout bream, CRFB10 and CRFB11 are expressed mainly in spleen and gill, while CRFB10 and CRFB11 in *T. nigroviridis* are not expressed in the same tissues, with CRFB11 expressed in brain and in a complementary manner among different tissues (Lutfalla et al., 2003). Further study is certainly required to clarify the expression pattern and function of CRFB10 and CRFB11. Distinctively, CRFB8/IL-20R1 shows high transcription level in mucosal tissues, such as intestine and gill (Lutfalla et al., 2003), and a similar situation was also observed in blunt snout bream. In rainbow trout, the expression pattern of the two paralogues, CRFB8a and CRFB8b of CRFB8/IL-20R1 are relatively similar (Monte et al., 2015). In grass carp, CRFB16/IL-20R2 was highly expressed in gills and skin, and the infection with *Flavobacterium columnare* and grass carp reovirus (GCRV) resulted in down-regulation of CRFB8 and CRFB16 in different tissues (Dang et al., 2023). But, the undetected expression of CRFB16 in blunt snout bream in the present study is unknown and requires further investigation. On the other hand, structural modeling showed that key residues involved in IL-20 interaction between grass carp and human are highly conserved, indicating evolutionarily conserved signal transduction and function in fish and mammals (Dang et al., 2023).

CRFB14/IL-22RA1 was predominantly expressed in mucosal tissues of yellow catfish (*Pelteobagrus fulvidraco*) and mandarin fish (Huo et al., 2021; Jiang et al., 2018), and CRFB9/IL-22RA2/IL-22BP transcripts increased only in IL-22-stimulated intestinal cells (Huo et al., 2021). In mammals, IL-22BP binds to IL-22 with high affinity, then blocking its binding to receptor complexes, IL-22RA1 and IL-10R2 (Kotenko et al., 2001). Analogically, IL-22BP has an inhibitory effect on IL-22 in mandarin fish, and is highly expressed in gill, intestine, pyloric caecum and other mucosal tissues. In addition, yeast two-hybrid assay confirmed the interaction between IL-22 and IL-22BP (Huo et al., 2019a). Noteworthily, three CRFB9 transcripts, CRFB9a, CRFB9b and CRFB9c, and two CRFB14 transcripts, CRFB14a and CRFB14b were identified in blunt snout bream, and were expressed in brain, trunk kidney and intestine except CRFB9b, respectively. However, the function of these transcripts needs to be investigated in future.

In summary, sixteen different CRFB members, CRFB1, CRFB2, CRFB4-17, were identified in blunt snout bream, with the presence of three CRFB9, and two CRFB14, that is CRFB1, CRFB2, CRFB4, CRFB5, CRFB6, CRFB7, CRFB8, CRFB9a, CRFB9b, CRFB9c, CRFB10, CRFB11, CRFB12, CRFB13, CRFB14a, CRFB14b, CRFB15, CRFB16 and CRFB17. The composition of more CRFB members in a teleost fish may provide clues to understand possible receptor-ligand interaction from an evolutionary point of view.

Data availability

Data will be made available on request.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dci.2023.104725.

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