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Research paper

Genome-wide identification, phylogeny, and expression of fibroblast growth genes in common carp

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ABSTRACT

Fibroblast growth factors (FGFs) are a large family of polypeptide growth factors, which are found in organisms ranging from nematodes to humans. In vertebrates, a number of FGFs have been shown to play important roles in developing embryos and adult organisms. Among the vertebrate species, FGFs are highly conserved in both gene structure and amino-acid sequence. However, studies on teleost FGFs are mainly limited to model species, hence we investigated FGFs in the common carp genome.

We identified 35 FGFs in the common carp genome. Phylogenetic analysis revealed that most of the FGFs are highly conserved, though recent gene duplication and gene losses do exist. By examining the copy number of FGFs in several vertebrate genomes, we found that eight FGFs in common carp have undergone gene duplications, including FGF6a, FGF6b, FGF7, FGF8b, FGF10a, FGF11b, FGF13a, and FGF18b. The expression patterns of all FGFs were examined in various tissues, including the blood, brain, gill, heart, intestine, muscle, skin, spleen and kidney, showing that most of the FGFs were ubiquitously expressed, indicating their critical role in common carp.

To some extent, examination of gene families with detailed phylogenetic or orthology analysis verified the authenticity and accuracy of assembly and annotation of the recently published common carp whole genome sequences. Gene families are also considered as a unique source for evolutionary studies. Moreover, the whole set of common carp FGF gene family provides an important genomic resource for future biochemical, physiological, and phylogenetic studies on FGFs in teleosts.

1. Introduction

Fibroblast growth factors (FGFs) are pleiotropic signaling molecules that are found in organisms from invertebrates to humans and control cell proliferation, migration, differentiation, and homeostasis. To date, the genes encoding FGFs have been identified in multicellular organisms ranging from *Caenorhabditis elegans* to humans (Goldfarb, 1990; Itoh and Ornitz, 2004). There are only two FGF genes found in *C. elegans*, whereas 22 FGF genes have been identified in humans and mice, indicating that the FGF gene family greatly expanded during the evolution of primitive metazoa to vertebrates. At least two major expansions of the superfamily have been recognized: the first expansion increased the number of FGFs from one or a few archeo-FGFs to eight proto-FGFs, prototypic of the eight subfamilies. The second expansion, which took place during the euchordate evolution, is associated with genome duplications (Popovici et al., 2005).

Between vertebrate species, FGFs are highly conserved in both gene structure and amino-acid sequence. A number of the vertebrate genomes including the rat, *Rattus norvegicus* (Gibbs et al., 2004), puffer fish, *Fugu rubripes* (Aparicio et al., 2002), and zebrafish, *Danio rerio*, have been completely sequenced and annotated, and orthologs of most of the human FGFs have been identified in these genomes. These findings indicate that no FGF gene was acquired in the mammalian lineage after the divergence of mammals from non-mammalian vertebrates. Comparisons of mammal genes with those of teleost fish have shown that in teleosts, including zebrafish, there are often two homologs of the mammalian equivalent. This suggests that there has been an additional whole genome duplication (WGD) shortly before the teleost radiation. Rapid gene loss occurred after the WGD event, as

Abbreviations: FGFs, fibroblast growth factors; WGD, whole genome duplication; JTT, Jones–Taylor–Thornton; SPR level 5, Subtree-Pruning–Regrafting — Extensive; TM, transmembrane domain; LCR, low complexity region; TS, teleost-specific; FHF, FGF homologous factors; Has, human; Dre, zebrafish; Gga, chicken; Xla, frog; Cca, common carp.

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most teleost species had returned to diploid status, for instance, only ~20% of the zebrafish genes still retain duplicated status in the genome (Aparicio et al., 2002).

There are 26 FGF genes in the zebrafish genome, which is comparable to 22 FGF genes in the human genome. To date, several FGFs have been isolated and well characterized in zebrafish, including FGF3 (Kiefer et al., 1996), FGF4 (Grandel et al., 2000), FGF6 (Postlethwait et al., 1998), FGF8 (Furthauer et al., 1997; Reifers et al., 1998), FGF10 (Ng et al., 2002), FGF17 (Reifers et al., 2000), FGF18 (Liu et al., 2002; Draper et al., 2003), and FGF24(Fischer et al., 2003). These genes show unique spatial–temporal expression pattern and may be implicated in various developmental processes during the development of zebrafish embryos. In the adult organism, FGFs are homeostatic factors and function in tissue repair and response to injury. When inappropriately expressed, some FGFs can contribute to the pathogenesis of cancer. A subset of the FGF family, expressed in adult tissue, is important for neuronal signal transduction in the central and peripheral nervous systems.

Common carp, *Cyprinus carpio*, one of the most significant aquaculture fish species, is widespread all over the word, especially, in Europe and Asia. Great efforts have been made in recent years to develop genomic resources for common carp, including a large number of expressed sequence tags (Christoffels et al., 2006), bacterial artificial chromosome end sequences (Xu et al., 2011a), comprehensive transcriptome obtained by RNA sequencing (Ji et al., 2012; Jiang et al., 2014), single nucleotide polymorphism (Xu et al., 2014a), and genetic and physical maps (Xu et al., 2011b; Zhao et al., 2013). The common carp whole genome sequences have recently been published (Xu et al., 2014b). It is known that the common carp genome is an allotetraploid genome, which had experienced an additional WGD round compared with most other teleosts (Zhang et al., 2008). Therefore, the complexity of the tetraploid genome and gene duplications may cause misidentification in assembly and annotation. Examination of gene families with phylogenic or orthology analysis would verify the whole genome sequences' assembly and annotation (Liu et al., 2013), as well as illustrate gene fates post the recent WGD event. As the FGF genes have been well characterized in mammalians as well as some model teleost species, we decided to characterize the FGF gene family in the common carp genome, and provide new insight into the gene evolution of the tetraploid genome (Satou et al., 2003). We identified 35 FGF genes in the common carp genome, and performed phylogenetic analysis across multiple representative vertebrate species. The gene nomenclatures based on phylogenetic topologies and sequence similarities assigned gene names to each of the 35 FGF genes in common carp. Moreover, the FGF gene expression profiles in common carp were examined in nine typical tissues, providing useful inference for function differentiation. The study on the FGF gene family not only validated the accuracy of the common carp whole genome sequences' assembly and annotation, but also provided valuable information for studies on development, evolution and genetics of common carp.

2. Materials and methods

2.1. Identification of common carp FGF genes

All available FGF genes of zebrafish (*D. rerio*) downloaded from Ensembl (http://asia.ensembl.org/index.html) were used as queries to search against the common carp available genomic resources including whole genome sequences, transcriptome sequences and cDNAs by BLAST searches to acquire candidate genes with the E value set as 1e − 5. Then, reciprocal BLAST searches were conducted using the candidate common carp FGF genes as queries, to verify the veracity of the candidate genes. Additionally, the coding sequences were confirmed by BLAST searches against the NCBI non-redundant protein sequence database (nr). The full-length amino acid sequences and the partial sequence coding for the conserved domains were used in the phylogenetic analysis. The FGF proteins from other organisms were retrieved from the Ensembl genome database (Release 75) for phylogenetic analysis, excluding of partial sequences.

2.2. Gene characterization

To characterize the genes' structures and compare them with their orthologs in the human and zebrafish genome, we first performed exon–intron structure analysis 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, which were further confirmed by conserved domain prediction by BLAST.

2.3. Phylogenetic analysis

To annotate the FGF genes, phylogenetic analysis was conducted with reference FGF proteins from zebrafish and human, and other representative vertebrate species. For nomenclatures of the common carp FGF, whenever possible we followed that of zebrafish because zebrafish is the most closely related model species to the common carp. Multiple protein sequences were aligned by ClustalW with default parameters. A neighbor-joining (NJ) phylogenetic tree of several types of typical vertebrate FGFs (Fig. 1) was constructed using the online tool, ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), with the default settings of Pairwise Alignment and Multiple Sequence Alignment. To further prove the validity of the NJ phylogenetic tree, we performed maximum likelihood analysis by MEGA6 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 trees were constructed using MEGA6 with Subtree-Pruning–Regrafting — Extensive (SPR level 5) as the LM Heuristic Methods. Each gene member assignment of common carp FGF protein was determined by phylogenetic analysis with FGF proteins from zebrafish and human. Separate phylogenetic analyses were conducted per family using the same methodology with more representative vertebrate species including zebrafish, chicken, frog, stickleback, mouse and human (Fig. S1).

2.4. Gene nomenclature

The FGF ortholog genes of common carp were named based on their phylogenetic topologies, as well as their most related zebrafish genes. First, the subfamilies and gene members were determined for each common carp FGF ortholog based on the phylogenetic clades (e.g. FGF1, FGF2). Then, the closely related zebrafish FGF genes were assigned to each common carp FGF orthologs. The FGF genes were named after their closely related zebrafish genes. When more than one copy of a common carp FGF gene was clustered with certain zebrafish FGF genes, Roman letter suffixes were added to each copy (for instance, FGF6a-1, FGF60a-2, FGF6b-1 and FGF6b-2). The name of each FGF gene in surveyed species is listed in Table 1 and Table 2.

2.5. Tissue expression profiling of FGF genes

Total RNA from various adult common carp tissues (blood, brain, gill, heart, intestine, muscle, skin, spleen, kidney) was extracted using TRIzol reagent (Life Technologies, Grand Island, NY, USA), and cDNA was synthesized by RT-PCR using the SuperScript III Synthesis System (Life Technologies). The β-actin gene was used as an internal positive control, with a forward primer (5′-TGCAAAGCCGGATTCGCTGG-3′) and a reverse primer (5′-AGTTGGTGACAATACCGTGC-3′). The PCR program comprised an initial denaturation step (2 min at 94 °C), followed by 30 cycles of denaturation (30 s at 94 °C), annealing (30 s at 60 °C) and extension (20 s at 72 °C), and a final elongation step (5 min at 72 °C). The PCR products were separated by gel electrophoresis (1.5% agarose

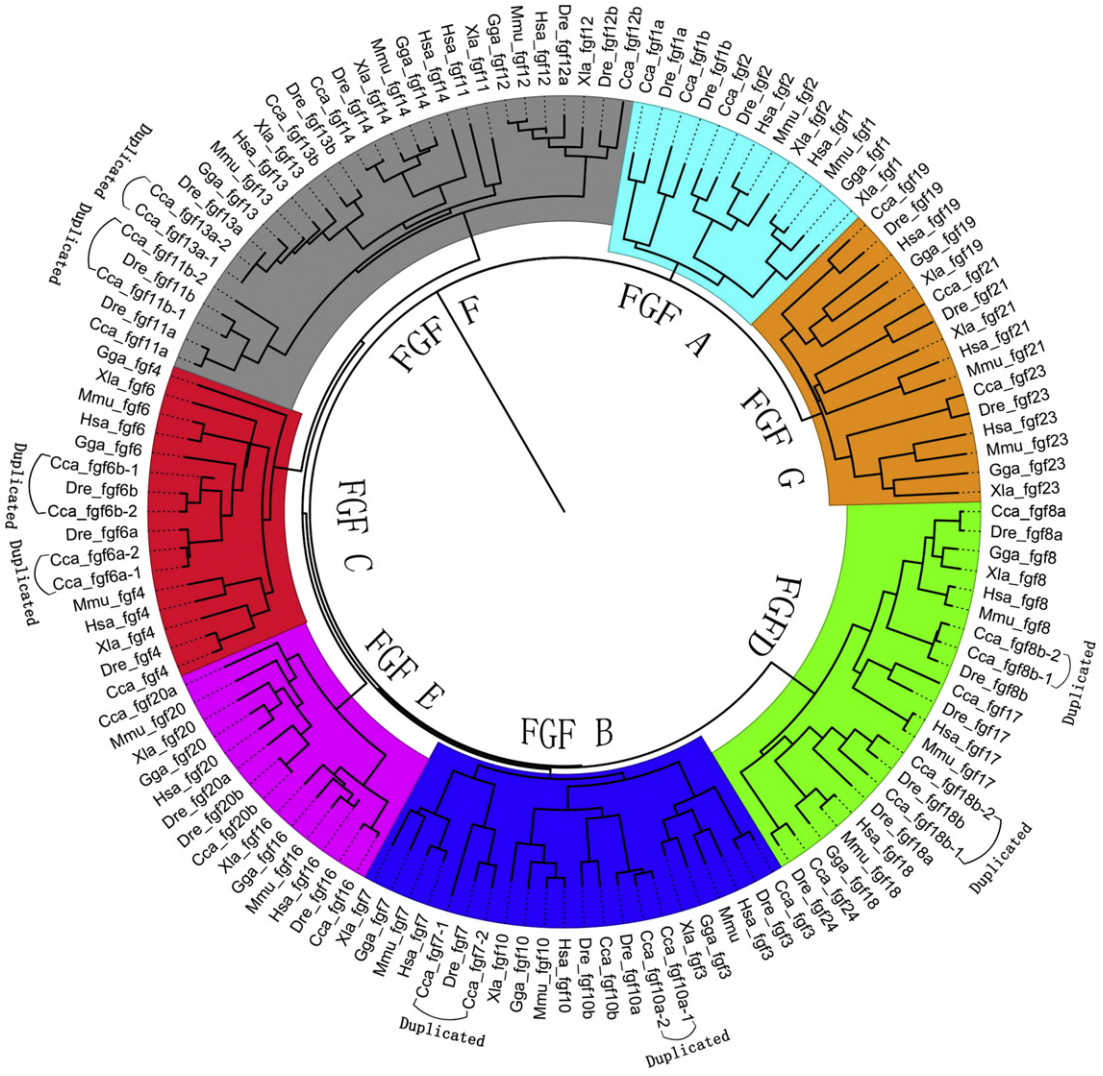

Fig. 1. Phylogenetic tree of the FGF gene family. Neighbor-joining-based phylogenetic tree of FGF protein sequences. Including several types of typical vertebrates: human (Has), zebrafish (Dre), chicken (Gga), frog (Xla) and common carp (Cca). FGF families are labeled from A to H.

gel at 150 V) in the presence of ethidium bromide and visualized under ultraviolet light.

3. Results and discussion

3.1. Identification of FGF genes in common carp

We identified a total of 35 FGF genes in the common carp genome using all available genomic resources, including FGF1a, FGF1b, FGF2, FGF3, FGF4, FGF6a-1, FGF6a-2, FGF6b-1, FGF6b-2, FGF7-1, FGF7-2, FGF8a, FGF8b-1, FGF8b-2, FGF10a-1, FGF10a-2, FGF10b, FGF11a, FGF11b-1, FGF11b-2, FGF12b, FGF13a-1, FGF13a-2, FGF13b, FGF14, FGF16, FGF17, FGF18b-1, FGF18b-2, FGF19, FGF20a, FGF20b, FGF21, FGF23 and FGF24 (Table 1). All the coding sequences of the FGF genes were deposited to the DDBJ database with the continuous accession numbers, LC026492–LC026527 (Table 1). Detailed information on their corresponding genomic sequences, coding sequences and number of exons is summarized in Tables 1 and 2. The structures of these FGF genes were then characterized and compared with the closely related model species zebrafish. There are 13 FGF genes with the same exon–intron organization compared with their homologous FGFs in zebrafish, while the others present various differences (Fig. S2 and Table 2).

The functional domains of the 35 genes were predicted, as shown in Fig. S3, demonstrating that all the FGFs possess one FGF domain. It has been revealed that the FGF domain functions on mitogens that stimulate growth or differentiation of cells of mesodermal or neuroectodermal origin. Therefore, this family plays essential roles in patterning and differentiation during vertebrate embryogenesis, and has neurotrophic activities. It also contains a number of transmembrane domains and a low complexity region. All of the 35 members possess one FGF domain, six (FGF6a-1, FGF6a-2, FGF10a-1, FGF10a-2, FGF10b, FGF18b-2) of which have one transmembrane domain, and seven (FGF3, FGF6b-2, FGF7-2, FGF10b, FGF14, FGF20b, FGF24) of which have an LCR (Fig. S1).

3.2. Phylogenetic analysis of fibroblast growth factors

The phylogenetic analysis revealed that the FGF genes of common carp were clustered with their respective homologs from other species (Fig. 1 and Fig. S1), indicating that all genes in the FGF gene family are highly conserved. The phylogenetic analysis is usually used as one of the most important evidence for gene annotation and nomenclature for non-model species, as described previously (Liu et al., 2013). In this study, the phylogenetic topologies provided us with strong evidence for correctly naming the 35 common FGF genes. As revealed by the phylogenetic tree, similarly to previous reports, there are seven major clades of the FGF gene family: FGF1 and FGF2; FGF10, FGF7 and FGF3; FGF6 and FGF4; FGF24, FGF18, FGF17 and FGF8; FGF16 and FGF20; FGF11, FGF12, FGF13 and FGF14; and FGF21, FGF19 and FGF23

Table 1
Summary of the FGF family in the common carp genome.

Gene name	Genomic length (bp)	CDS (na)	CDS (aa)	CDS status	Accession no.
FGF1a	2425	444	147	Complete	LC026492
FGF1b	969	387	128	Complete	LC026493
FGF2	4496	468	155	Complete	LC026494
FGF3	2284	771	256	Complete	LC026495
FGF4	2510	576	191	Complete	LC026496
FGF6a-1	4624	618	205	Complete	LC026497
FGF6a-2	2837	618	205	Complete	LC026498
FGF6b-1	2347	207	68	Partial	LC026499
FGF6b-2	3011	630	209	Complete	LC026500
FGF7-1	5680	696	231	Partial	LC026501
FGF7-2	3397	588	195	Complete	LC026502
FGF8a	4523	633	210	Complete	LC026503
FGF8b-1	2304	597	198	Complete	LC026504
FGF8b-2	3051	573	190	Complete	LC026505
FGF10a-1	14,157	606	201	Complete	LC026506
FGF10a-2	13,310	987	328	Complete	LC026507
FGF10b	13,192	594	197	Complete	LC026508
FGF11a	3181	744	247	Complete	LC026509
FGF11b-1	20,841	585	194	Partial	LC026510
FGF11b-2	15,092	462	153	Partial	LC026511
FGF12b	10,961	600	199	Partial	LC026512
FGF13a-1	31,341	978	325	Complete	LC026513
FGF13a-2	29,962	582	193	Complete	LC026514
FGF13b	15,883	603	200	Complete	LC026515
FGF14	53,210	747	248	Complete	LC026516
FGF16	5013	612	203	Complete	LC026517
FGF17	3862	729	242	Complete	LC026518
FGF18b-1	4985	348	115	Partial	LC026519
FGF18b-2	2335	642	213	Complete	LC026520
FGF19	2594	633	210	Complete	LC026521
FGF20a	1522	441	146	Partial	LC026522
FGF20b	1184	627	208	Complete	LC026523
FGF21	1125	432	143	Partial	LC026524
FGF23	1701	777	258	Complete	LC026526
FGF24	8265	618	205	Complete	LC026527

(Fig. 1)(Kim, 2001; Satou et al., 2002; Itoh and Ornitz, 2004). Namely, there are seven subfamilies of the FGF genes in common carp, corresponding to previously designated names as follows: FGF A, FGF B, FGF C, FGF D, FGF E, FGF F, FGF G (Table 3). We did not identify FGF genes for subfamily FGF H, which exists in chordates (Popovici et al., 2005). The major branches have a similarly long length, suggesting that they appeared very early in evolution.

3.3. Gene duplications and losses of FGFs in common carp

WGD is one of the major drivers that shaped the evolutionary history of many vertebrates. Ohno has suggested that two rounds of large-scale gene duplication had occurred early in vertebrate evolution (Ohno, 1970), and a number of studies of comparative analysis of various gene clusters provided solid evidences in support of Ohno's hypothesis (Postlethwait et al., 1998; McLysaght et al., 2002; Dehal and Boore, 2005). An additional round of duplication, also named teleost-specific (TS) WGD, or the 3R WGD (Aparicio, 2000; Aparicio et al., 2002), took place in the common ancestor of all extant teleosts. As a result of genome duplication, teleost fish usually have two paralogous copies for many genes, while only one ortholog is present in tetrapods.

It has been hypothesized that an additional WGD (the 4R WGD) occurred in common carp during its evolution. A number of studies have provided various evidences in support of the 4R duplication event in common carp (David et al., 2003; Zhang et al., 2013; Zhao et al., 2013). The comprehensive estimation based on whole genome datasets suggests that the latest WGD event occurred around 8.2 MYA (Xu et al., 2014b).

Following a WGD event, lineage-specific gene duplication and gene loss are frequently observed during evolution, which provides useful information for gene evolution studies. We examined and compared the copy numbers of FGF genes in representative vertebrate genomes. There are at least 16 FGF genes in common carp with duplicated copies comparable to the eight FGF genes in the zebrafish genome, including FGF6a, FGF6b, FGF7, FGF8b, FGF10a, FGF11b, FGF13a and FGF18b (Fig. 1 and Supplementary Table S1). Most likely, these duplications resulted from the latest 4R WGD. The gene loss did not occur on these members post-WGD event. There are four FGF6 genes in common carp, two in zebrafish and only one in higher vertebrates such as human, which provide the typical example instance for the multiple rounds of WGD. The TS WGD (3R) doubled the FGF6 gene in the zebrafish genome, which contains FGF6a and FGF6b. The common carp specific 4R WGD further doubled the FGF6 genes in common carp, which contains FGF6a-1, FGF6a-2, FGF6b-1 and FGF6b-2. In addition to the duplication, we observed a significant portion of FGF genes that retained only one copy in most of the surveyed teleost species, including FGF2, FGF3, FGF4, FGF12, FGF14, FGF 16, FGF17, FGF19, FGF 21, FGF23 and FGF24. Obviously, there may be critical selective pressure on these single copy genes which does not allow more than one copy of certain genes in the genome. Therefore, the gene loss may occur quickly after a WGD event, to maintain the balance of gene expression and gene regulation. In addition, comparing with zebrafish genome we have not found FGF5, FGF9, FGF12a, FGF18a and FGF22 in the common carp genome. FGF15 is absent in all investigated teleost genomes, which suggests that FGF15 may be a newly emerged FGF gene in higher vertebrates, or resulted from gene loss in ancient teleosts. Zebrafish genome still has a relative large gene set (~26,000 genes) comparing with many other diploid teleost (20,000–23,000 genes) (Kasahara et al., 2007; Howe et al., 2013; McGaugh et al., 2014). Thus, zebrafish genome may still retain significant number of replicated genes that generated from the 3rd WGD, as an instance, we observed duplicated FGF genes, e.g. FGF8a and FGF8b, FGF13a and FGF13b, in the zebrafish genome. Comparing with zebrafish genome, common carp FGF genes are further expanded due to the extra 4th WGD. For instance, we observed three FGF8 and three FGF13 genes in the genome (Table 2). Regarding gene losses, it may occur in FGF gene family in common carp post the latest WGD as those identified 35 FGF genes are much less than our expectation, however, we also suspect another possibility that imperfect genome assembly and annotation lead to the "gene losses", especially on such a tetraploidized genome of common carp.

3.4. Tissue expression profiles of FGF genes of common carp

During embryonic development, FGFs have diverse roles in regulating cell proliferation, migration and differentiation. The FGF family contains many secreted signaling proteins that are expressed in nearly all tissues and serve essential roles in the earliest stages of embryonic development, during organogenesis, and in the adult, where they function as homeostatic factors that are important for tissue maintenance, repair, regeneration, and metabolism. Compared with the number of known FGF genes in embryos, the number of FGF genes identified in the adult organism is very limited, especially in teleosts. Given the unexpected expansion of the FGF gene family in common carp, it was of interest to examine how many of these genes are actually expressed, and to determine the expression pattern of these genes to identify potential function differentiation. To this END, we conducted RT-PCR using gene-specific primers to examine the expression pattern of each gene in various common carp tissues. As shown in Fig. 2, FGF gene families exhibit unique tissue-specific expression. In general, most of the FGF genes are widely expressed, but with a relatively high expression level in the blood, brain, gill, heart, intestine, spleen and kidney (Fig. 2).

Establishing the gene expression pattern, together with the information on orthologs from model species should provide functional inferences, with the understanding that the function of lineage specific genes can be distinct depending on the living environments under different selection pressures (Liu et al., 2013). Functional inferences for FGF genes that have undergone duplications or losses in teleost

Table 2
Structure and localization of both common carp and zebrafish FGF genes.

Common carp			Zebrafish		
FGF genes	Chromosome/scaffold no.	No. of exons	FGF genes	Chromosome/scaffold no.	No. of exons
FGF1a	Chromosome 1	3	FGF1a	Chromosome 14	3
FGF1b	Scaffold	2	FGF1b	Chromosome 21	2
FGF2	Scaffold	2	FGF2	Chromosome 14	3
FGF3	Scaffold	3	FGF3	Chromosome 7	3
FGF4	Scaffold	3	FGF4	Chromosome 7	3
			FGF5	Chromosome 5	3
FGF6a-1	Chromosome 49	3	FGF6a	Chromosome 4	3
FGF6a-2	Chromosome 49	3			
FGF6b-1	Scaffold	2	FGF6b	Chromosome 4	3
FGF6b-2	Chromosome 2	3			
FGF7-1	Chromosome 43	5	FGF7	Chromosome 18	3
FGF7-2	Chromosome 35	3			
FGF8a	Chromosome 37	4	FGF8a	Chromosome 13	5
FGF8b-1	Chromosome 10	3	FGF8b	Chromosome 1	6
FGF8b-2	Chromosome 2	4			
			FGF9	Chromosome 9	3
FGF10a-1	Scaffold	4	FGF10a	Chromosome 21	3
FGF10a-2	Chromosome 41	5			
FGF10b	Scaffold	3	FGF10b	Chromosome 5	3
FGF11a	Scaffold	2	FGF11a	Chromosome 7	5
FGF11b-1	Scaffold	5	FGF11b	Chromosome 10	5
FGF11b-2	Chromosome 36	3			
			FGF12a	Chromosome 2	5
FGF12b	Chromosome 30	4	FGF12b	Chromosome 15	5
FGF13a-1	Scaffold	7	FGF13a	Chromosome 14	6
FGF13a-2	Chromosome 28	5			
FGF13b	Scaffold	5	FGF13b	Chromosome 10	6
FGF14	Chromosome 27	4	FGF14	Chromosome 9	5
FGF16	Scaffold	3	FGF16	Chromosome 14	3
FGF17	Chromosome 3	5	FGF17	Chromosome 8	5
			FGF18a	Chromosome 14	5
FGF18b-1	Chromosome 21	2	FGF18b	Chromosome 10	5
FGF18b-2	Chromosome 22	3			
FGF19	Scaffold	3	FGF19	Chromosome 7	3
FGF20a	Chromosome 1	3	FGF20a	Chromosome 1	3
FGF20b	Scaffold	3	FGF20b	Chromosome 14	5
FGF21	Chromosome 5	2	FGF21	Scaffold	4
			FGF22	Chromosome 22	3
FGF23	Scaffold	3	FGF23	Chromosome 4	3
FGF24	Scaffold	4	FGF24	Chromosome 14	5

fish are very interesting because they potentially underlie the adaptations.

We observed that most of the FGF genes were widely expressed in three tissues (brain, skin and spleen). Thirty-one of the 35 members were expressed in the brain, implying their important roles in the nervous system regulation (Smallwood et al., 1996; Xu et al., 2000; Turner et al., 2012). However, FGF6b, FGF7, FGF8, FGF20a and FGF20b were widely expressed in all the nine investigated tissues with only a slight difference between two duplicates (if duplicated genes do exist), suggesting their universal functions in common carp.

The expression profiles of the common carp FGF genes, as shown in Fig. 2, suggest that most of them have similar gene functions as confirmed in many model species. For instance, FGF1 is involved in various biological functions including angiogenesis, wound healing, feeding regulation, neurogenesis, and neuroprotection. Previous studies have demonstrated that FGF1 contributes to the neuroprotective activity in neuron injury or neurological disorders in animal models and human patients (Chen et al., 2015). FGF2, which is present in both neurons and glial cells, has previously been reported to have multiple neural-promoting effects on the developing and the adult nervous system of mice and other mammals (Adeeb and Mortazavi, 2014). According to the higher expression level of FGF1 and FGF2 in carp skin and brain, we can speculate that they may play similar functions in common carp. FGF6's tissue expression is essentially restricted to developing and adult skeletal muscle (Fiore et al., 2000; Itoh and Ornitz, 2008). Consistently, FGF6a-1, FGF6a-2, FGF6b-1 and FGF6b-2 were observed in the muscle of common carp. FGF11, FGF12, FGF13 and FGF14 were previously named FGF homologous factors (FHF) 3, FHF1, FHF2 and FHF4, respectively, and are predominantly expressed in the nervous system in higher vertebrates (Zhang et al., 2012). FGFs 11–14 lack signal peptides, remain and function within cells in a receptor-independent manner. These FGFs might be intracellular components of a tissue-specific protein-kinase signaling module and seem to share structural, but not functional, homology with other FGFs (Schoorlemmer and Goldfarb, 2001). They are predominantly expressed in common carp brain, suggesting their conserved gene functions across all vertebrates.

We also observed significant gene expression differences compared with previous studies on model species. For instance, it has been suggested that FGF3 and FGF19 are confined to mouse and chicken hindbrain boundary cells (Sela-Donenfeld et al., 2009). However, FGF3 gene was expressed in the spleen, skin and muscle, and FGF19 was expressed in blood in common carp, indicating the different gene functions in teleosts compared with higher vertebrates. A study on human

Table 3
Seven subfamilies of FGF genes in common carp.

FGF A	FGF B	FGF C	FGF D	FGF E	FGF F	FGF G
FGF1	FGF3	FGF4	FGF8	FGF16	FGF11	FGF19
FGF2	FGF7	FGF6	FGF17	FGF20	FGF12	FGF21
	FGF10		FGF18		FGF13	FGF23
			FGF24		FGF14	

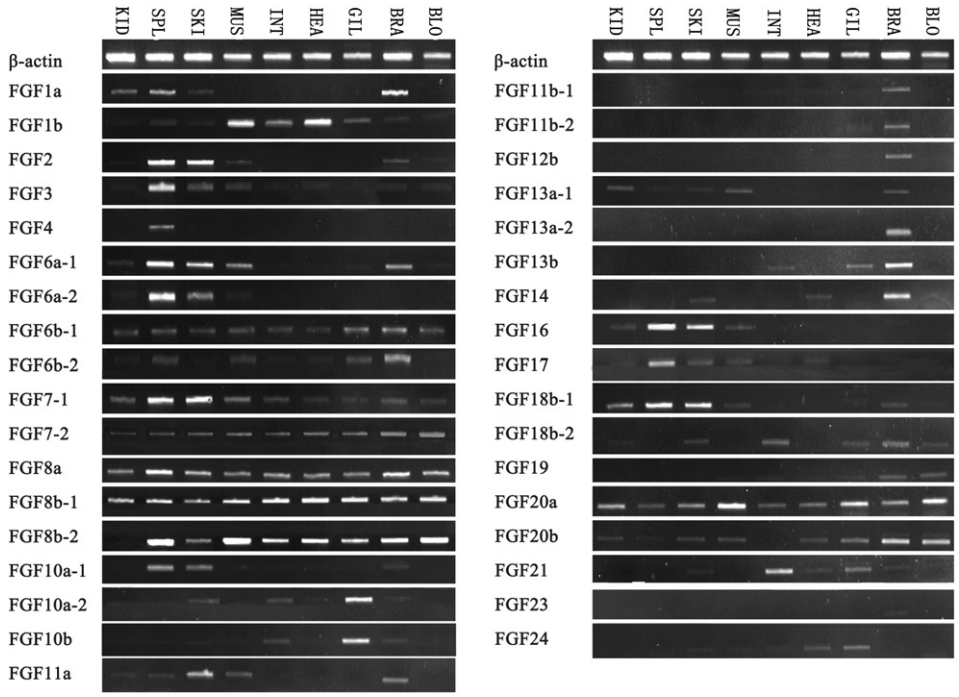

Fig. 2. RT-PCR based expression analysis of common carp FGF genes. Expression of the different FGF genes in various common carp tissues. The amplification of β-actin was used as an internal control. Gene names are indicated on the left. The tissues are: KID (kidney), SPL (spleen), SKI (skin), MUS (muscle), INT (intestine), HEA (heart), GIL (gill), BRA (brain), BLO (blood).

FGF4 has suggested that it was a risk factor for craniosynostosis (Grillo et al., 2014); however, the FGF4 gene of common carp was solely expressed in spleen.

Additionally, we observed significant expression differences in those duplicated FGF genes, providing evidence for gene subfunctionalization post-WGD event. For instance, there are significant expression differences between FGF1a and FGF1b. FGF1a is highly expressed in the kidney, skin, spleen and brain, while FGF1b is highly expressed in the muscle, heart, intestine and gill. Similarly, we observed expression differences between FGF10a-1 and FGF10a-2, and between FGF13a-1 and FGF13a-2. Most likely, the ancestral gene was capable of performing all functions and was expressed broadly in the tissues, while the descendant duplicate genes only perform partial functions and are specifically expressed in certain tissues. The functional divergence of duplicated genes may avoid potential adaptive conflicts (Conrad and Antonarakis, 2007).

4. Conclusions

A total of 35 FGF genes were identified in the common carp genome. Phylogenetic analysis allowed annotation of these FGF genes. Our results showed that the majority of the FGF genes were well conserved through evolution. Clear orthologous relationships were established for the majority of the FGF genes, enabling functional inference on the common carp transporters. Most of the FGF genes were ubiquitously expressed in common carp, but highly expressed in the tissues that are more likely to be involved with FGF genes, indicating the critical roles of this gene family in nervous system regulation. However, the detailed function of each gene needs further study. The whole set of FGF genes provides essential genomic resources for future biochemical, toxicological, physiological and evolutionary studies on common carp.

Author contributions

PX conceived the study. LJ, SZ and PX wrote the manuscript. CD and BC performed the bioinformatics analysis. JF and WP conducted the phylogenetic analysis. SM and KAG helped with the manuscript preparation. All authors have read and approved the final version of the manuscript. The authors declare that there is no conflict of interest.

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