Genome-Wide Comparative Analyses of Pigmentation Genes in Four Fish Species Provides Insights on Fish Skin Color Patterning

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Abstract: Although it is well known that vertebrates universally use the chromatophore to control their body color patterns, the difference of chromatophore types within vertebrates were observed: mammals have one single chromatophore type while fish could possess six types of chromatophores. To better understand the molecular mechanisms underlying the chromatophore diversity in fish, the genomic resources and data of four fish species, Cynoglossus semilaevis, Danio rerio(zebrafish), Oreochromis niloticus and Paralichthys olivaceus, including whole-genome sequence, annotation, and transcription data, were exploited. A total of 61 pigmentation-related genes were identified in all studied species and classified into six groups including the Mcr gene family group, which plays the most important role in melanocortin system. The numbers of each pigmentation-related gene family members are similar across all four species, except Mc3r gene is only found in zebrafish genome. Comparative analysis of pigmentation-related genes structures and phylogenetic analysis provide a clear orthologous relationship for all Mcr genes. For Danio rerio and Cynoglossus semilaevis, using the skin samples from different developmental stages, the transcriptome analyses revealeda diverse expression pattern across all pigmentation-related genes and Dbh and Hbxd3a genes highly co-expressed with multiple pigmentation-related genes. The STRING analysis of pigmentation-related proteins in zebrafish demonstrates that five proteins, corticotrophin releasing hormone, oxytocin-like protein, opioid receptor (delta), prepronociceptin α and β have strong interactions with pigmentation-related proteins. This work provides a better understanding of compositions of pigmentation-related genes in four fish species and provides insights on the far-reaching genetic mechanism of chromatophore-controlled pigmentation.

1. Introduction

Fish melanophores only produce eumelanin, but no pheomelanin[1][2]. Fish skin pigmentation is controlled by the melanocortin system, which is consisted with some endogenous agonists, melanocortin receptor subtypes (MC1,2,3,4,5R), the proopiomelanocortin (pomc) gene that provides melanocortin peptides, and the agouti signaling protein family (Asip1, Asip2, Agrp1 and Agrp2)[3][4]. The interaction between endogenous agonists (such as alpha and beta melanocyte stimulating hormone (Msh)[5][4] and adrenocorticotropic hormone (Acth)) results in diversified pigmentation patterns in fish. Meanwhile, some other genes involved in fish pigmentation have been reported, such as the microphthalmia-associated transcription factor (Mitf)[6], a putative cation exchanger Slc24a5[7], and the Slc7a11 gene involved in pheomelanin synthesis[8].

Most of studies on fish pigment patterning were conducted in teleost fish species, which represent over 96 percent of all living fish species and about half of all living vertebrates[9]. Momentary stimulus causes physiological color change upon pigment organelle aggregation or dispersion within skin chromatophores. It has been reported that melanin is produced and deposited in specific organelles called melanosomes in fish and mammals[10]. Interestingly, fish only synthesize eumelanin while pheomelanin has not beenfound in fish[11][2].

Five melanocortin receptor proteins (MC1-5R),are known to mediate the diverse actions of

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melanocortins through their spatial distributions, ligand affinities and specificities[Ref?]. Although Mc1r gene is a well known key pigmentationregulator in mammals, its physiological functions in fishare not well defined. In addition to those melanocortin receptors, there are a few other components in the melanocortin system, such as the endogenous agonists alpha and beta melanocyte stimulating hormone (Msh), melanocortin peptides derived from the proopiomelanocortin (pomc), adrenocorticotropic hormone (Acth), and the endogenous antagonists of the agouti signaling protein family (Asip1, Asip2, Agrp1 and Agrp2). All above-mentioned components in their cellular context constitute the melanocortin system, by which fish use to regulate the pigment production and dispersion.

In this study, a total of 61 pigmentation-related protein sequences were identified by a genome-wide search against four teleost species' genomes, Cynoglossussemilaevis(Cse), Danio rerio(Dre), Oreochromisniloticus(Oni) and Paralichthys olivaceus(Pol). Their phylogenetic relationships were determined.The transcriptome analysesofDanio rerioshowed different expressionprofiling in pigmentation-related genes .To better understand the pigment regulation network, the expression levels of pigmentation-related geneswere compared among different tissues.

2. Materials and methods

2.1 Genome-wide identification of pigmentation-related genes

To identify 13 target pigmentation-related genes in these four fish species, we first used the gene names and taxonomy information to search Uniprot protein database (http://www.uniprot.org). Please see the detailed method and criteria used in Supplementary Table 2) to identify all related protein sequences. The sequence and annotation data of all four fish genomes were downloaded from NCBI GenBank database. The target protein sequences from Uniprotwere used as query to search against the annotated protein sequence dataset for each species using local BLASTP program(NCBI blast+ v2.6.0) with E-value cutoff of 1e-10. We also used the amino acid sequences of each pigmentation-related gene fromzebrafish genome to search against the genome assemblies of Cynoglossussemilaevis, Daniorerio, Oreochromisniloticus and Paralichthys olivaceus via TBLASTN program with E-value cutoff of 1e-10.All BLAST results were intergrated to provide thetop five candidates for each query sequence followed by further filtering using the criteria as following, 1) the length range of the sequence of homologous proteins; 2)similarity and 3) gene structure. A total of 61 pigmentation-related genes (Supplementary Table 1) and their corresponding encoding protein sequences were obtained from four fish species as the final version. The identified pigmentation-related genes were renamed according to the general principle.The amino acid sequences of all identified pigmentation-related proteins from four fish species were first classified into different groups based on their sequence homology and annotation.

2.2 Multiple sequence alignment and phylogenetic analysis

MSA for each of six groups were conducted using ClustalX software(v2.1) with default settings. Each MSA result was saved as MSF format and fed into Jalview software (v2.10.1; http://www.jalview.org) for manual editing. The edited MSF file was imported into PAUP*4 (Build157; http://paup.phylosolutions.com) for phylogenetic tree construction using Branch-swapping algorithm in Distance Method (Bootstrap replicates=3000, seed=111 with other default settings). The final tree file was stored in Newick file format.

2.3 Public transcriptomic data mining

Public zebrafish skin RNA-Seq data was downloaded from NCBI GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66362) and the RPKM data of all skin samples without retenone treatment were used to identify differentially expressed genes across these samples using the DESeq software setting P value <0.05 as a threshold (http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html). The corresponding RPKM

data for target genes were also extracted and compared. We worked on existing data on zebrafish, which is relatively complete in public database, and examined the expression profiles of pigmentation-related genes in Daniorerio. The data in PRJNA276667 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA276667) was analyzed. Meanwhile,

2.4 Co-expression analysis of pigmentation-related genes and the network study of proteins encoded by pigmentation-related genes in zebrafish

Using the co expression database COXPRESdb (http://coxpresdb.jp) of multi-model species, 16 target genes of zebrafish were searched for co-expressed genes by querying Entrez Gene ID, and the best hit of 100 co-expressed genes were listed out. Six target genes (Pomcb, Agrp1, Slc7a11a, Slc7a11b, Slc24a5, Asip1) could not found co-expressed genes in this study. The remaining 10 target genes with co-expressed genes were statistically analyzed (see complete co-expression data, Supplementary table 8). We also employed String to build the protein interaction network for proteins encoded by pigmentation-related genes.

3. Results

3.1 Genome-wide identification of pigmentation-related genes in four fish species(Cynoglossus semilaevis, Daniorerio, Oreochromisniloticus, and Paralichthys olivaceus)

A total of 15 pigmentation-related genes were identifiedin Cynoglossus semilaevis (Table 1). The Agrp, Asip, MiTFgenes were found to have two paralogs, respectively (Agrp1/Agrp2, Asip1/Asip2, MiTFa/MiTFb. The Pomcgene has three copies (Pomca/b/c). Most of other pigmentation-related genes are single-copy in the genome. The Mc3rgene was not foundin Cynoglossus semilaevis. Table 2 lists the genome-wide search results of six groups of all 61 target genes that were identified in all four fish species' genomes (please see detailed information for all genes in Supplementary table 1). No Agrpgene was found in Paralichthys olivaceus genome. Agouti signaling protein gene contains two copies in Cynoglossus semilaevis and Paralichthys olivaceus, contrasting to one copy inOreochromis niloticus and Daniorerio.It is worth noting thatthe Mc3rgene could not be identified in three fish genomes but in Daniorerio genome.

Table 1 The information of pigmentation-related genes in Cynoglossussemilaevis

Gene Name	GeneID (NCBI)	Linkage Group	Genomic Position	Protein_ID	Length(aa)	No. Exons
Agrp1	103378815	Chr5	6678771..6680785	XP_008308383.1	135	4
Agrp2	103396135	Chr20	4232466..4236566	XP_008332333.1	115	4
Asip1	103386778	Chr11	19849938..19852383	XP_008319411.1	124	3
Asip2	103377057	Chr3	6984889..6995098	XP_008305912.1	135	3
Mc1r	103379178	Chr5	14316804..14318904	XP_008308872.1	338	1
Mc2r	103388887	Chr13	19201646..19202751	XP_008322312.1	300	2
Mc4r	103377490	Chr3	16166181..16168647	XP_008306547.1	316	1
Mc5r	103388888	Chr13	19207827..19227811	XP_008322313.1	346	5
MiTFa	103386307	Chr11	11089087..11092667	XP_008318720.1	377	8
MiTFb	103385328	Chr10	17137395..17164062	XP_016891654.1	507	10
Pomca	103389827	Chr14	15956240..15959516	XP_008323644.1	388	5
Pomcb	103380837	Chr7	5037661..5043144	XP_008311170.1	238	3
Pomcc	103393769	Chr18	222737..224118	XP_008329069.1	178	3
Slc24a5	103378833	Chr5	6955975..6962253	XP_016887920.1	512	9
Slc7a11	103382740	Chr8	20532593..20539016	XP_008313852.1	484	13

Table 2 Copies number of pigmentation-related genes among four fish species

Name	Description	Dre	Oni	Cse	Pol	ALL
agrp	agouti related neuropeptide	2	2	2	0	6
asip	agouti signaling protein	1	1	2	2	6
mc1r	melanocortin 1 receptor	1	1	1	1	4
mc2r	melanocortin 2 receptor	1	1	1	1	4
mc3r	melanocortin 3 receptor	1	0	0	0	1
mc4r	melanocortin 4 receptor	1	1	1	1	4
mc5r	melanocortin 5 receptor	2	1	1	1	5
mitf	microphthalmia-associated transcription factor	2	2	2	2	8
pomc	proopiomelanocortin	2	3	3	3	11
slc24a5	solute carrier family 24 (sodium/potassium/calcium exchanger), member 5	1	2	1	1	5
slc7a11	solute carrier family 7 (anionic amino acid transporter light chain)	2	2	1	2	7
Total		16	16	15	14	61

3.2 Comparative analyses and phylogenetic relationships of 61 pigmentation-related genesamong four fish species

The result of multiple sequence alignment (MSA) of the full-length MC1/2/3/4/5R proteins is shown in Figure 1. The MSA results for the other 5 groups of homologous proteins are shown in Supplementary Figure 1A-1E.The MSA of MC1-5R proteins showed a high similarity in their sequences but not at their N-terminal. The conserved domains across MCR proteins among different species suggest their functional significance. The MSA of agouti gene family also suggested that the four fish species have variations in this gene family. This result indicates that four agouti genes in teleosts arerelatively less conservative than melanocortin receptors (Mcrs).Similarly, the MITF and POMC groups are less conservative than SLC7A11Band SLC24A5.

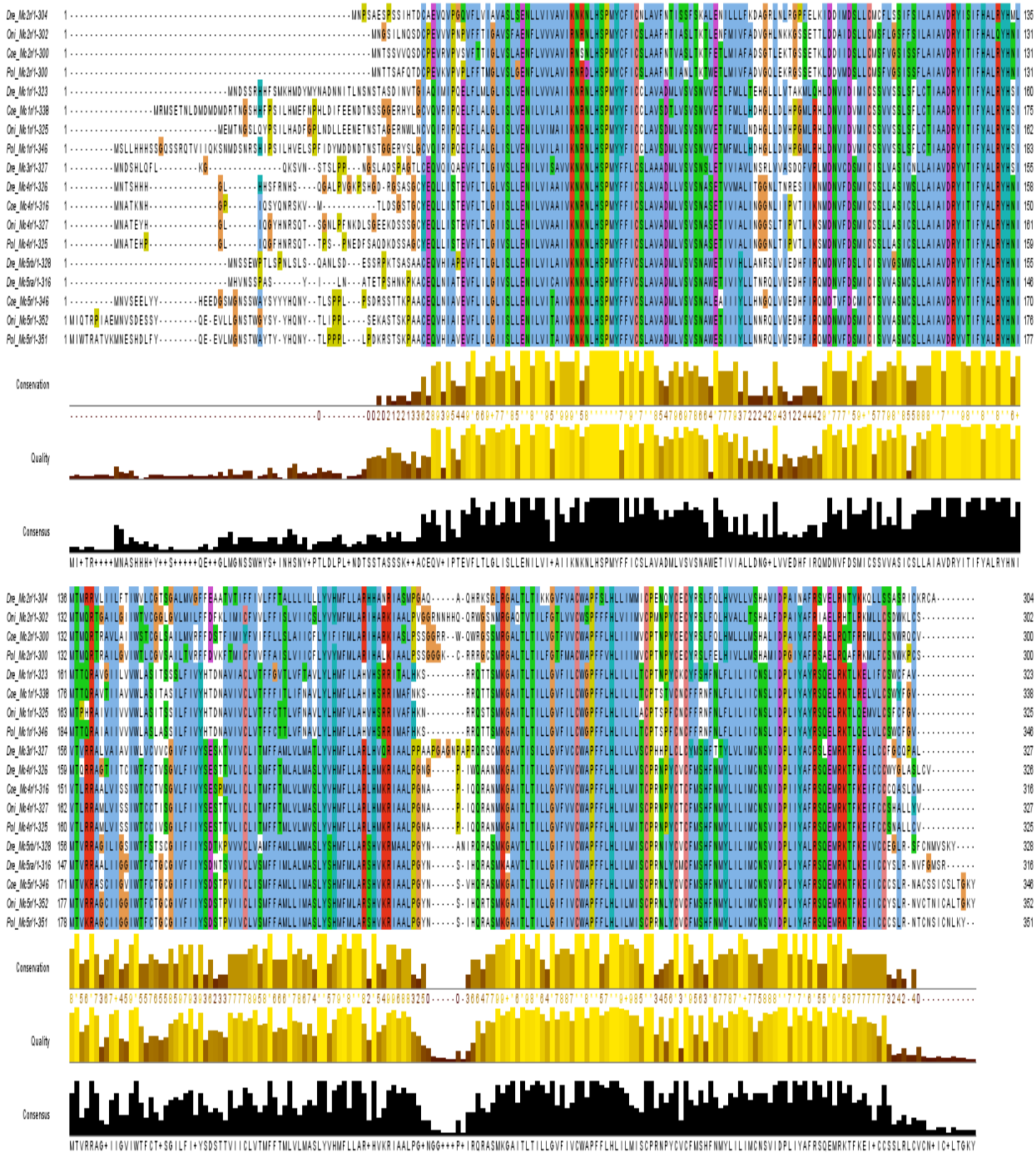


Figure 1 Results of the full length Mc1-5r homologous protein MSA in four fish species

The phylogenetic tree was constructed for each group using ClustalX and the gene structures were drawn along with each tree (Figure 2 and Supplementary Figure 2A-2E). The unrooted tree shows that all Mcr genes are divided into five clusters as expected. The Mc3r, Mc4r and Mc5r are more closely related to each other than the Mc1r and Mc2r with the observation that Mc2rappears more distantly related to the rest of Mcr proteins. Regarding gene structures, Mc5r genes have more introns than the other Mcr genes in three fish species (Cynoglossussemilaevis, Oreochromisniloticus and Paralichthysolivaceus). The other pigmentation-related genes, such as Mitf, Slc7a11a, Slc24a5, contain multiple introns and exons in all four fish species genomes. Finally, the phylogenetic trees from Mcr genes in four different fish species suggested that divergence of Mcr genes happened earlier than the speciation of these bony fishes in this study.

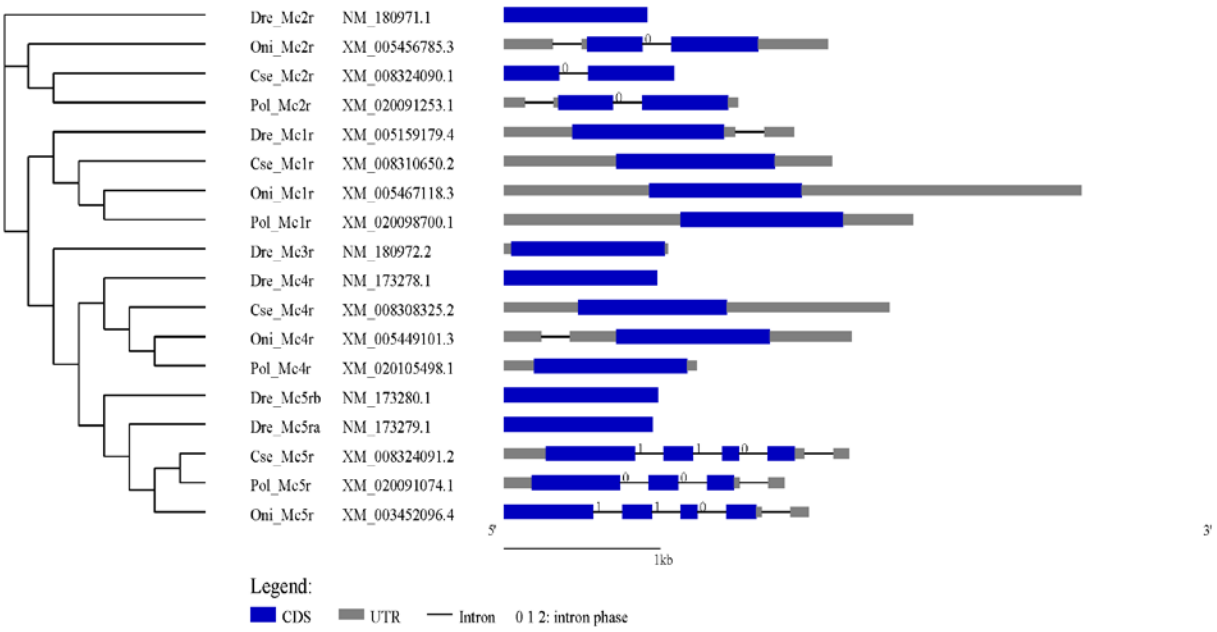


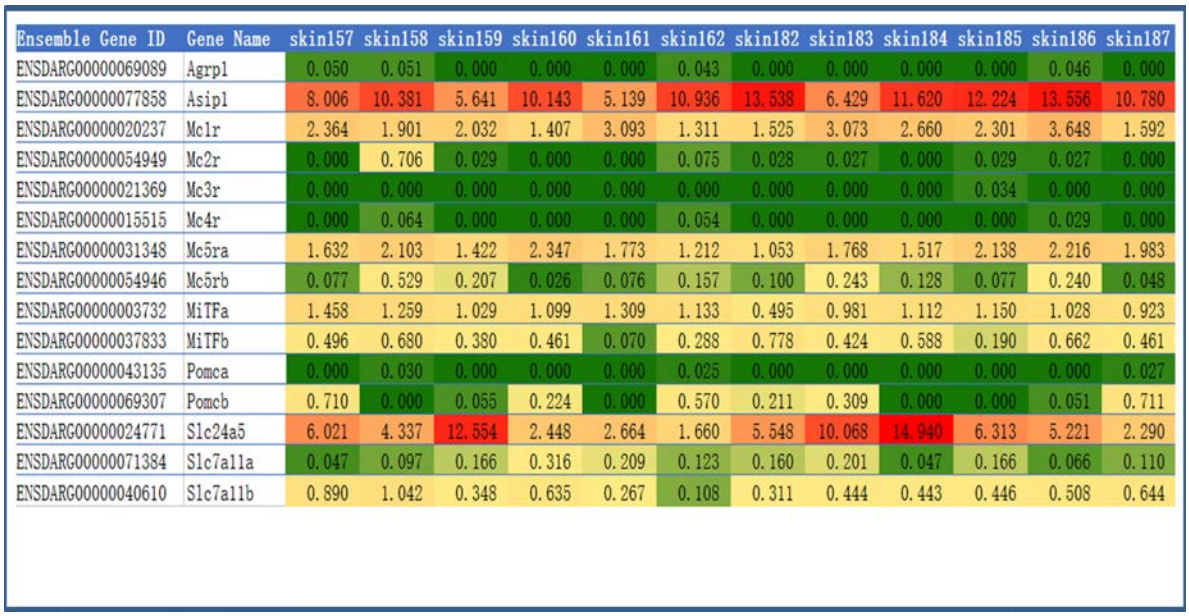
Figure 2 Mc1r-mc5r genes structures and phylogenetic trees in in four fish species

3.3 Skin transcriptome analysis of pigmentation-related genes at different developmental stages in Danio rerio

We selected four developmental stages of skin tissue for transcription comparative analysis (including 3 replicates and a total of 12 samples, Figure 3). The RPKM images of 15 pigmentation-related genes (except Agrp2) were calculated according to the RNA-seq data. Asip1 and Slc24a5 genes were highly expressed in all skin tissue samples. In Mcr gene family, the expression intensities of Mc2r, Mc3r, and Mc4r genes are weaker than that of Mc1r and Mc5ra/b.Mc1r; and Mc5ra and Mitfa presented moderate expression. The expression of Slc24a5 showed great elevation in two stages. The differential expressions of 22 pigmentation-related genes among developmental stages were analyzed. The results were shown in Supplementary table 2-7). Two comparison groups, 12 months (Age)8 weeks (Culture duration) group vs 36months _3 weeks and 12 months _8 weeks vs 36 months_8 weeks, had the most differentially expressed genes, while the number of differentially expressed genes between 12_3 and 36_3 group was only 170, and the number of differentially expressed genes between 36_3 and 36_8 group was 210(Table 3). Of the 16 pigmentation-related genes of zebrafish, only the Slc24a5 gene (ENSDARG00000024771) was differentially expressed in 36_3 and 12_8.

Table 3 Number of gene expression differences between different developmental stages in zebrafish

——	12_8	36_3	36_8
12_3	547	170	829
12_8	-	1253	1177
36_3	-	-	210



Ensemble Gene ID	Gene Name	skin157	skin158	skin159	skin160	skin161	skin162	skin182	skin183	skin184	skin185	skin186	skin187
ENSDARG00000069089	Agrp1	0.050	0.051	0.000	0.000	0.000	0.043	0.000	0.000	0.000	0.000	0.046	0.000
ENSDARG00000077858	Asip1	8.006	10.381	5.641	10.143	5.139	10.936	13.538	6.429	11.620	12.224	13.556	10.780
ENSDARG00000020237	Mc1r	2.364	1.901	2.032	1.407	3.093	1.311	1.525	3.073	2.660	2.301	3.648	1.592
ENSDARG00000054949	Mc2r	0.000	0.706	0.029	0.000	0.000	0.075	0.028	0.027	0.000	0.029	0.027	0.000
ENSDARG00000021369	Mc3r	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.034	0.000	0.000
ENSDARG00000015515	Mc4r	0.000	0.064	0.000	0.000	0.000	0.054	0.000	0.000	0.000	0.000	0.029	0.000
ENSDARG00000031348	Mc5ra	1.632	2.103	1.422	2.347	1.773	1.212	1.053	1.768	1.517	2.138	2.216	1.983
ENSDARG00000054946	Mc5rb	0.077	0.529	0.207	0.026	0.076	0.157	0.100	0.243	0.128	0.077	0.240	0.048
ENSDARG00000003732	MiTFa	1.458	1.259	1.029	1.099	1.309	1.133	0.495	0.981	1.112	1.150	1.028	0.923
ENSDARG00000037833	MiTFb	0.496	0.680	0.380	0.461	0.070	0.288	0.778	0.424	0.588	0.190	0.662	0.461
ENSDARG00000043135	Pomca	0.000	0.030	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.027
ENSDARG00000069307	Pomcb	0.710	0.000	0.055	0.224	0.000	0.570	0.211	0.309	0.000	0.000	0.051	0.711
ENSDARG00000024771	Slc24a5	6.021	4.337	12.554	2.448	2.664	1.660	5.548	10.068	14.940	6.313	5.221	2.290
ENSDARG00000071384	Slc7a11a	0.047	0.097	0.166	0.316	0.209	0.123	0.160	0.201	0.047	0.166	0.066	0.110
ENSDARG00000040610	Slc7a11b	0.890	1.042	0.348	0.635	0.267	0.108	0.311	0.444	0.443	0.446	0.508	0.644

Figure 3 RPKM images of 15 genes expression profiling at four developmental stages in Daniorerio according to the RNA-SEQ data

3.4 Co-expression analysis and network study of proteins encoded by pigmentation-related genes in Danio rerio.

The pigmentation-related genes with multiple co-expressed genes were listed in table 4. In general, 10 out of 16 pigmentation-related genes were found co-expressed genes in Daniorerio; six pigmentation-related genes, including Pomcb, Agrp1, Slc7a11a, Slc7a11b, Slc24a5, and Asip1, were not found co-expression hits.In this study, Dopamine beta-hydroxylase gene (Dbh) and Homeobox D3a (Hbxd3a) gene showed co-expression with multiple pigmentation-related genes. Dbh (GeneID:30505), co-expressing with seven pigmentation-related genes in our study,was reported to express in noradrenergic nerve terminals of the central and peripheral nervous systems, as well as in chromaffin cells of the adrenal medulla in other studies. Additionally, Hoxd3a (GeneID:30349) was found to co-express with six pigmentation-related genes in this study.

Table 4 Co-expression analysis of pigmentation-related genesin Daniorerio

Entrez Gene ID	Gene Name	Gene Function	No. Co-expressed genes	Co-expressed genes
30505	dbh	Dopamine beta-hydroxylase (dopamine beta-monooxygenase)	7	MiTFb;Mc4r;Mc5ra;Mc5rb;Mc1r;Mc2r;Mc3r
30349	hoxd3a	Homeobox D3a	6	MiTFb;Mc5rb;MiTFa;Mc1r;Mc3r;Pomca
415181	psmc1b	Proteasome (prosome, macropain) 26S subunit, ATPase, 1b	5	Mc4r;Mc5ra;Mc1r;Mc2r;Mc3r
797346	spint1b	Serine peptidase inhibitor, Kunitz type 1 b	4	Mc5ra;Mc5rb;Mc2r;Mc3r
402987	cga	Glycoprotein hormones, alpha polypeptide	3	Mc5rb;Mc1r;Pomca
445387	ddx51	DEAD (Asp-Glu-Ala-Asp)	2	Mc1r;Mc2r

		box polypeptide 51		
100001537	dok2	Docking protein 2	2	MiTFa;Mc1r

Finally, we used String to build the protein interaction network for 15 pigmentation-related gene-encoded proteins.Agrp2 (ENSDARG00000099781) was not retrieved in database. The protein interaction network was shown in Figure 4, which suggested strong interactions among 15 proteins encoded by pigmentation-related genes. Meanwhile, these 15 proteins showed strong interactions with five other proteins encoded by genes, Corticotropin-releasing encoding neuropeptide hormone gene (crh; ENSDARP00000038676), oxytocin-like gene (oxtl; ENSDARP00000062884), Prepronociceptin a/b (pnoca/b; ENSDARP00000035567/ENSDARP00000032986) gene and Opioid receptor gene (oprd1a; ENSDARP00000122239). The evidence of the interaction to five nervous system proteins was shown in Table 5.

Table 5 The network interaction of proteinscoding with 15 retrieved genes.

Gene name	Gene description	Experiments	Databases	Textmining	Homology	Score
crh	Corticotropin releasing hormone (162 aa)	●	●	●	●	0.996
oxtl	Oxytocin-like (154 aa)	●	●	●	●	0.972
oprd1a	Opioid receptor, delta 1a (374 aa)	●	●	●	●	0.968
pnoca	Prepronociceptin a (276 aa)	●		●	●	0.966
pnocb	Prepronociceptin b (233 aa)			●	●	0.966

"●",means supported

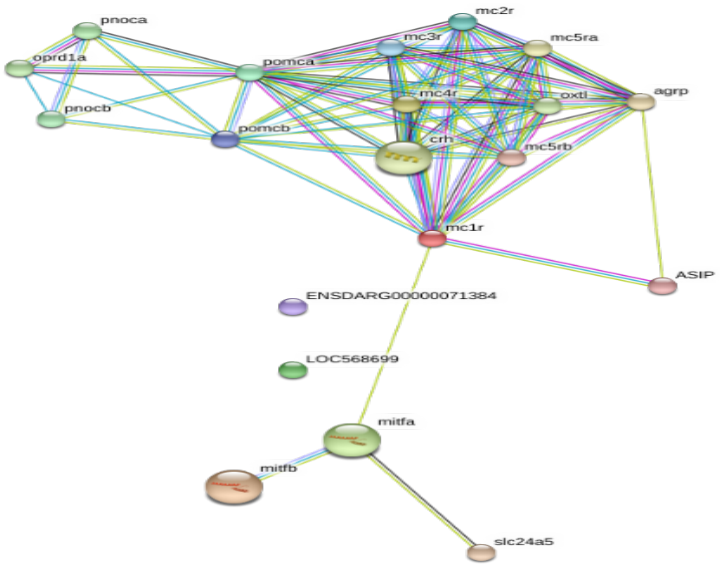


Figure 4 The network interaction of proteinscoding with 15 retrieved pigmentation-related genes in Daniorerio

4. Discussion

Pigment pattern is mainly caused by the organized distribution of pigment cells, which is regulated by pigmentation-related genes via the color signal pathway. It has been extensively reported that physiological and morphological colorchanges were controlled by the sympathetic nervous system (norepinephrine) and endocrine system (including alpha melanocyte stimulating hormone, alpha Msh, and melanin concentrating hormone, MCH)[12]. In our study,13 pigmentation-related genes involved in melanin generating/regulating system were used as queries to search homologous genes and proteins in four genome-annotated databases of Cynoglossussemilaevis, Daniorerio, Oreochromisniloticus and Paralichthys olivaceus. We found that each individual fish genome contains one or two copies of Mc1r, Mc2r, Mc4r and

Mc5r,whereas Mc3r is only found in Daniorerio genome. The lack of Mc3r in three genomes is similar to pufferfish (Tetraodon nigroviridis), which was reported to have four receptors and lack Mc3r [13]. The composition of Mc5r in four genomes showed slightly difference with zebrafish that has six receptors with additional copy of Mc5r[14]. In mammals, the Mc1r gene that is conferred a key mechanism for vertebrate pigment phenotypes, is mainly expressed in skin tissue. In fish, Mc1r stimulates melanin production and melanosome dispersion in melanophores. MC1r genes are positively related to the formation of melanin in skin tissues,the high expression of MC1r genes promotes the formation of melanin .The expression of MC1r in the skin was locally consistent with the pigment content. The copy number, affinity, specificity of Mcr gene is far from defined and appears to be species-specific. Former study has indicated that mcr genes might be vertebrate specific since there is no Mcr gene found in the urochordates, which are the closest living relatives to vertebrates[15]. Phylogenetic trees from Mcr genes in four different fish species suggested that the forming subtypes of Mcr gene has occurred prior to the speciation. It merits mention that two Pleuronectiformes fishes share the ancestry in Carangimorpharie with Perciformes. In our study, Cynoglossus semilaevis and Paralichthys olivaceus are belong to the same order of Pleuronectiformes, Daniorerio belongs to the order of Cypriniformes, and Oreochromis Niloticus is in the order of Perciformes. Cse, Oni and Pol are closer than Dre, which is consistent with previous studies [16].All four fish species have the common ancestry in Osteogloss ocephalai that is belong to one of four teleost groups[16] .The copies of MCR paralogs found in some teleost are more likely originated from whole genome duplication that occurred during the early evolution.

The agouti gene family such as asip1, asip2, agrp, and agrp2, were described in previous study[17] . It has suggested that asip2 and agrp2 are duplicates from asip/asip1 and agrp1, respectively, resulting from teleost genome duplication (TGD) [17]. Our result of the copy numbers of the agouti gene family in four flatfishes is consistent with former study. Themultiple sequences alignmentsof agouti family genes showed the similarity of the four fish species. However, the four agouti family genes in teleosts arerelatively less conservative than te melanocortin receptors (Mcrs).Similarly, the MITF.POMC isrelatively less conservative than SLC7A11Band SLC24A5. The similar situations occurred in other fishes.

The skin pigmentation-related genes expression profiling of Daniorerio showed that TYRP1 and MC1R genes are positively related to the formation of melanin in fish skin,the high expression of TYRP1 and MC1R genes promoted the formation of melanin. In mammals, the melanin synthesis pathway via protein kinase A (PKA) is activated by the cAMP, and this pathway also involves the tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1)[18][19].The same mechanism occurs in teleosts in spite of a slight difference that the most widely known effect of alpha-MSH-MC1R in fish is in fact the dispersion of pigments in melanocytes[12]. Interestingly, melanosomes are dispersed by the effect of α-Msh also via a cAMP.

Dbh gene and Hbxd3a gene display co-expression with multiple pigmentation-related genes. Co-expressed with seven target genes, Dbh (Dopamine beta-hydroxylase,Dbh, GeneID:30505)encodes the only enzyme involved in the synthesis of small-molecule, membrane-bound norepinephrine, a neurotransmitter synthesized inside vesicles. Dbh is expressed in noradrenergic nerve terminals of the central and peripheral nervous systems, as well as in chromaffin cells of the adrenal medulla. Also,Homeobox D3a (Hoxd3a, GeneID:30349) ,which is closely related with the development of an important regulatory transcription factor,co-expressed with 6 target genes in this study. In addition to regulating the early developmental patterns of the central nervous system, the Hox gene also determines the differentiation and identity of the cells. Some studies suggest that Hox transcriptional factors regulate tissue regeneration and are associated with tumorigenesis. In fish,It can be speculated the Hox gene may be involved in the production and localization of pigments cells

5. Conclusions

Our work is the first study of genome-wide identification of pigmentation-related genes within four fish species by examining the transcriptome data of their skin tissues. A total of 61

pigmentation-related genes from four fish species, Cynoglossus semilaevis, Daniorerio, Oreochromisniloticus and Paralichthys olivaceus, were identified and classified into six groups. In particular, we observed an expression pattern of pigmentation-related genes, which contributes to diversity patterns of fish skin color pathway. Together, our work provides a better understanding of compositions of pigmentation-related genes in four fish species and may have far-reaching genetic mechanism of chromatophore-controlled pigmentation.

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