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The Genetic Basis of Skin Color and Body Shape of Domesticated Purse Red Carp Revealed by Comparative Transcriptome Analysis

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Authors' Contribution
YJ conceived and designed the project, and wrote the article. SZ performed the experiments and analyzed the data. YG analyzed the data. JX helped in data analysis. MH performed the experiments. PX helped in preparation of the article.

Key words
Transcriptome, Purse red carp, Skin color, Body shape, Genetic basis.

ABSTRACT

Purse red carp is an economic important and well-known domesticated variety of common carp in China. During a long history of domestication, purse red carp displays consumer-favored morphological traits such as red skin color and purse-shaped body form. However, the underlying genetic basis is less studied. In the present study, we utilized the powerful approach of comparative transcriptome analysis via high throughput sequencing, and examined transcriptome profiles of purse red carp by comparing with Yellow River carp, of which the skin color and body shape are quite similar to wild carp. A total of 434 million reads was generated, resulted in 203,590 assembled contigs, which was used as the reference sequences. Comparing the skin and blood transcriptome files showed that 2,767 unigenes were significantly differentially expressed, including 1,663 genes up-regulated and 1,806 down-regulated in purse red carp. Moreover, gene pathway analysis of the differentially expressed genes was conducted. We proposed the skin color and body shape depended upon at least four pathways including melanin biosynthesis, TGF-beta signaling, Wnt signaling and MAPK signaling pathways. We obtained candidate genes that may affect the morphological characteristics of purse red carp resulted from the long history of domestication, including *TYRP1b*, *PMELa*, *KITa*, *BMP8a*, *ACVR2aa*, *TGFB1a*, *TGFB3*, *TGFBR2*. Understanding the molecular mechanisms underlying the biological characteristics of purse red carp will advance our knowledge of the genetic differentiation between different common carp varieties caused by domestication, and accelerate the molecular selection of fish species with consumer-favored skin color and body shape.

INTRODUCTION

The common carp (*Cyprinus carpio*), is a significant freshwater fish widespread all over the world, especially in Eurasia. It is one of the longest cultured and the most domesticated fish species in the world (Balon, 1995). During such a long history of domestication, common carp has been introduced into various environments worldwide, resulting in numerous varieties and strains that display rich biodiversity and diverse morphological characteristics. Artificial selection is believed to be one of the most important evolutionary forces in the process of domestication (Xu *et al*., 2012), which could alter allele frequency and genetic diversity (Chistiakov and Voronova, 2009; Wang *et al*., 2010). Such genotypic variations contribute to phenotypic variations, mainly by modulating gene expressions. Therefore, it is important to understand the gene expression profiling underlying interesting traits resulted from domestication. However, the differential expression pattern of genes related to morphological traits caused by domestication is less studied.

Purse red carp (*Cyprinus carpio* var. Wuyuanesis) is a well-known domesticated variety of common carp, with about 800 years of cultivation history. It is widely used as breeding parents for crossbreeding and hybridization in Chinese aquaculture industry. The most significant

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morphological traits of purse red carp are its red skin color, purse-shaped body form with a ratio of 2.0–2.3 in body length/depth, and a shorter caudal peduncle length (Li *et al*., 2005). Phenotypic variation of morphological traits in fish would be controlled by genetic effect, environmental effect, or interaction of genotype and environment, but heritable traits such as skin color and body form were likely resulted from the action and interactions of many genes that act differentially during ontology. Skin color is a significant economic trait of common carp, serving as an important criterion for visually determining its quality and marketing value. It is necessary to understand the genetic effects from the selective or differential expression of genes. There were several studies that focused on the genetic basis of skin coloration in common carp, but none of them were studied on purse red carp by far. For instance, Wang *et al*. (2014) reported that 80% of pigmentation genes from zebrafish were also present in Oujiang color carp. *MC1R* gene was found involved in the development of black pigmentation in the ornamental Koi common carp (Bar *et al*., 2013). Our previous study of comparing the skin transcriptome files of Xingguo red carp with Yellow River carp had provided initial insights into the molecular mechanism underlying the skin pigmentation process (Jiang *et al*., 2014). Another important trait of common carp is body shape, which is associated with swimming abilities, anti-predator adaptations, and fish meat yields. Some factors such as growth hormone are known to play important roles in body size and body shape, however, the underlying genetic regulation is still poorly understood. Zhang *et al*. (2013) utilized a linkage map and identified 5 significant QTLs that affect body shape of common carp, but no related genes were reported.

In order to comprehensively understand the genetic basis underlying the morphological characteristics of purse red carp, we utilized the powerful approach of comparative transcriptome analysis via high throughput sequencing, and examined transcriptome profiles from skin and blood of purse red carp, by using Yellow River carp as control, since the skin color and body shape of Yellow River carp are quite similar to wild carp (Balon, 1995). They are brownish-black, and generally elongated and spindle shaped, with a ratio of 3.34±0.48 in body length/depth. We obtained candidate genes that may affect the morphological characteristics of purse red carp resulted from the long history of domestication. Understanding the molecular mechanisms underlying biological characteristics of purse red carp will advance our knowledge of genetic differentiation between different common carp varieties caused by domestication, and accelerate the molecular selection of fish species with consumer-favored skin color and body shape.

MATERIALS AND METHODS

Ethics statement

This study was approved by the Animal Care and Use committee of Centre for Applied Aquatic Genomics at Chinese Academy of Fishery Sciences. All fish were euthanized by using MS-222 before sampling, and all efforts were made to minimize suffering.

Fish sampling and RNA extraction

The purse red carp used in this study were provided by National Fish Hatchery of Purse Red Carp at Wuyuan County, Jiangxi, China, and the Yellow River carp were provided by Henan Academy of Fishery Sciences, Zhengzhou, Henan, China. All fishes were maintained in the laboratory at the same time and acclimatized for 4 weeks before experimental use. Six tissues from 18 individuals of each carp variety were collected, including brain, blood, gill, head kidney, skin and muscle. All tissues were immediately immersed into 10 ml RNAlater (Qiagen, Hilden, Germany), and stored at -80°C until RNA isolation. The total RNA was extracted from each tissue using TRIzol (Invitrogen, Carlsbad, CA, USA) with DNase I following the manufacturer's instructions. RNA quality was verified using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA), and visualized under ultraviolet light following gel electrophoresis in the presence of ethidium bromide. The high-quality RNA from each tissue was sequenced on an Illumina HiSeq 2000 platform.

Sequence data processing, de novo assembly and transcriptome annotation

CLC Genomics Workbench (CLC bio, Aarhus, Denmark) was used for raw sequencing data processing. Low quality reads with Q<20 and length below 20 bp were removed, and adaptor sequences were trimmed. All the cleaned reads assembled by the Trinity software with default parameters. The assembled contig sequences were filtered using CD-hit program (Fu *et al*., 2012) to reduce redundancy. The resulting contigs longer than 200 bp were considered as the final non-redundant transcripts and were used as the reference sequences for further comparative transcriptome study.

To better annotate the assembled transcriptome contigs, three public databases were used, including the Ensembl zebrafish protein database, the UniProt database and the NCBI *nr* database. BLASTx searches were performed with an E-value cutoff of 1E-10. Gene ontology (GO) analysis was conducted by using InterProScan (Zdobnov and Apweiler, 2001). The annotation output was categorized by cellular component, molecular function and

biological process at the 2nd level by importing annotation results into a web-based tool WEGO (Ye *et al*., 2006).

Differential gene expression analysis

The differential gene expression analysis was performed as previously described (Jiang *et al*., 2014), with slight modifications. Briefly, all the cleaned reads were mapped to the assembled reference transcriptome by Bowtie (Langmead *et al*., 2009). RSEM was then used to calculate and estimate gene or isoform abundances, and edgeR (Robinson *et al*., 2010) was used to normalize the expression level of each transcript in each sample. Transcripts with fold change values larger than 2 and *p* value lower than 0.05 were considered as significantly differential expressed genes and used for subsequent analysis.

Quantitative reverse transcription PCR (qRT-PCR) validation

qRT-PCR was used to validate the RNA-Seq results on randomly selected 15 genes. The beta-actin gene was used as an internal reference, and primers were designed as below: forward primer: 5′- TGCAAAGCCGGATTCGCTGG -3′; reverse primer: 5′- AGTTGGTGACAATACCGTGC -3′. Briefly, qRT-PCR was performed in optical 96-well plates on an ABI PRISM 7500 Real-time Detection System (Life Technology). The amplification was performed in a total volume of 15 µl, containing 7.5 µl 2X SYBR Green Master Mix reagent (Life Technology), 1 µl of cDNA (100 ng/µl), and 0.2 µl of 10 µM of each gene specific primer. The thermal cycling profile consisted of an initial denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. All reactions were set up in triplicates including the negative controls with no template. To assess PCR efficiency, five 10-fold serial dilutions of a randomly selected cDNA sample were used on both the target genes and the reference gene to assess the PCR efficiency. After the PCR, data were analyzed with ABI 7500 SDS software. The comparative CT method ($2^{-\Delta\Delta CT}$ method) was used to analyze the expression of the target genes. All data were given at levels relative to the expression of the beta-actin gene.

RESULTS AND DISCUSSION

Transcriptome sequencing, reference assembly and annotation

The long history of domestication enhanced several consumer-favorable traits of purse red carp such as red skin color, and purse-shaped body form. To comprehensively understand the differences of gene expression related to those morphological characteristics of purse red carp, we compared the transcriptome files of purse red carp with that of Yellow River carp. Yellow River carp originated from Yellow River basin. The morphological traits of Yellow River carp was quite similar to wild carp, with brownish-black skin color and elongated and spindle shape, made it a good reference carp variety for comparative transcriptome analysis in this study. First, we generated a reference sequences for subsequent analysis. Six tissues were collected and deep-sequenced, including brain, blood, gill, head kidney, muscle and skin. As shown in Table I, a total of 434 million paired-end reads were generated, of which 223 million were from purse red carp and 211 million were from Yellow River carp. After the removal of ambiguous nucleotides, short reads (length<20 bp) and low quality sequences (Q<20), a total of 427 million clean reads were retained for further analysis, with an average ratio of 98.5%. The number of clean reads ranged from 28824994 to 35809741, with outliers of 55377054 showing the stability of consistence on sampling, library preparation and sequencing.

Table I.- Summary of the raw sequencing data.

Fish strain/Tissue	Reads	Clean reads	Ratio (%)
Yellow River carp			
Brain	36,058,166	35,120,875	97.4%
Skin	36,899,438	35,809,741	97.0%
Gill	35,941,220	35,016,622	97.4%
Blood	35,114,692	34,267,222	97.6%
Head kidney	30,353,832	29,523,567	97.3%
Muscle	36,496,782	35,529,894	97.6%
Purse red carp			
Brain	39,758,052	39,606,472	99.6%
Skin	29,259,548	29,131,199	99.6%
Gill	55,615,924	55,377,054	99.6%
Blood	28,991,084	28,824,994	99.4%
Head kidney	35,118,242	34,979,388	99.6%
Muscle	34,145,310	34,020,856	99.6%

All clean reads were pooled and assembled into reference sequences. As shown in Table II, a total of 203590 contigs with length longer than 200 bp were generated, of which, 45712 contigs were longer than 1000 bp. The maximum length of the assembled contigs was 27233 bp. The N50 was 2050 bp and the average length was 892 bp.

To predict genes contained in the assembled contigs, BLASTx searches was performed against three protein databases, including the NCBI non-redundant (nr) database, Uniprot database and Ensembl zebrafish protein database, with an E-value cutoff of 1e-10. The number of contigs with significant hit against the three databases

were 28506, 21391, 22596, respectively (Table II). Cumulatively, 46657 assembled contigs had at least one significant hit against at least one of the three databases, allowing for the prediction of 29572 unique genes.

Table II.- Statistics of the assembled reference sequences.

Parameters	Value
Assembly	
No. of contigs (>200 bp)	203,590
No. of larger contigs (>1000 bp)	45,712
Maximum length (bp)	27,233
N50 (bp)	2,050
Average length (bp)	892
Annotation	
No. of contigs with Blast hit to nr	28,506
No. of contigs with Blast hit to UniProt	21,391
No. of contigs with Blast hit to zebrafish protein	22,596
No. of unique genes predicted	29,572
No. of contigs with GO terms	46,686
No. of unique genes with GO terms	14,114

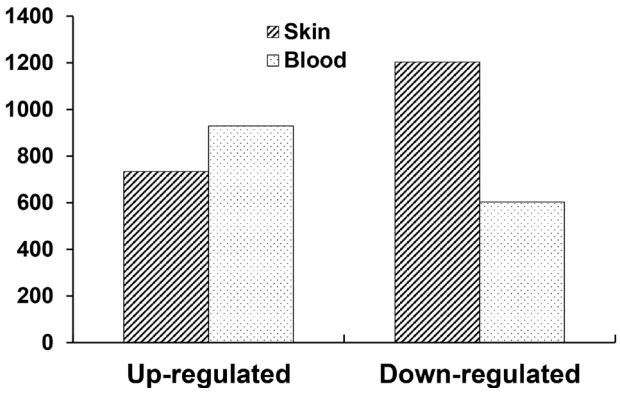

Fig. 1. The differentially expressed genes in the skin and blood between two common carp varieties. The y-axis represents the number of differentially expressed genes, and the x-axis represents different tissues.

Gene ontology (GO) annotation was then performed, of which, 14,114 genes were annotated with GO terms. A total of 42 GO terms were assigned, including 11 cellular component terms (26.2%), 21 biological process terms (50%), and 10 molecular function terms (23.8%). In the category of biological process, cellular process was the most predominant terms, accounting for 46.8% of the genes annotated in that term, and it was followed by metabolic process, accounting for 38.2%. While in the category of molecular function, binding was the most predominant term (67.8%), followed by catalytic activity (31.3%).

Identification of differentially expressed genes in purse red carp

In this study, we focused on the differentially expressed genes in the skin and blood transcriptome files. Based on the criteria of |fold change|≧2 and p value<0.05, a total of 2,767 unigenes showed significantly different expression level in at least one tissue, skin or blood. As shown in Figure 1, the total number of differentially expressed genes in skin (1937) is more than that in blood (1532). Comparing with Yellow River carp, there are 734 and 929 unigenes significantly up-regulated in skin and blood, respectively, while there are 1203 and 603 unigenes down-regulated in skin and blood, respectively.

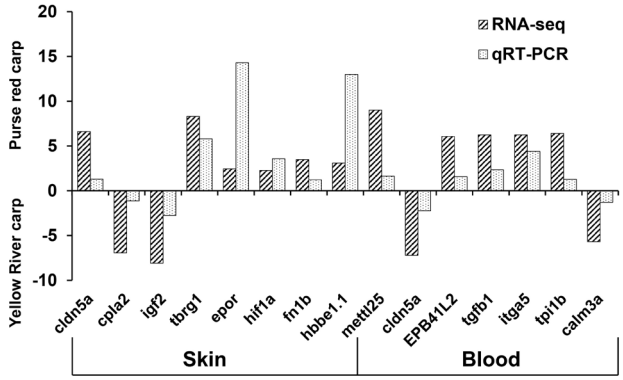

Fig. 2. Comparison of gene expression patterns obtained using comparative transcriptome analysis and qRT-PCR. Fold changes are expressed as the ratio of gene expression comparing purse red carp with Yellow River carp after normalization to β-actin.

In order to further validate the differentially expressed genes identified by comparative transcriptome analysis, qRT-PCR were performed on 15 randomly selected genes. Three biological replicates samples were used. Melting curve analysis showed that only a single amplified product was generated, indicating the specificity of the product. As shown in Figure 2, the direction of the expression pattern of all tested genes were in agreement with the results of comparative transcriptome analysis, indicating that no consistent bias existed for either method.

Enrichment analysis

We attempted to categorize all 2767 differentially expressed genes according to their potential functions by using gene annotation, GO enrichment analysis and gene pathway analysis. All genes were classified into different cellular, biological and functional gene ontologies by using InterProScan software. Comparing the GO annotation of differentially expressed genes with that of all population genes, the web-based program WEGO

revealed 25 significantly over-represented GO terms at the 2nd GO level that were enriched in purse red carp. Table III shows 10 representative over-represented GO terms, including pigmentation (GO:0043473), anatomical structure formation (GO:0010926), biological adhesion (GO:0022610), biological regulation (GO:0065007), cellular component biogenesis (GO:0044085), cellular component organization (GO:0016043), developmental process (GO:0032502), growth (GO:0040007), immune system process (GO:0002376), response to stimulus (GO:0050896). Our primary interest in this study was to detect expression signatures indicative of fish skin pigmentation and body shape, therefore, all genes contained in the anatomical structure formation, pigmentation, developmental process and growth GO categories were considered to be most informative for further pathway analysis.

Table III.- Ten representative GO terms in the GO enrichment results of significantly differentially expressed genes identified in purse red carp compared to Yellow River carp. The "Count Ratio" column indicates study count ratio/population count ratio; the study count ratio is the ratio of genes associated with the GO term to all genes in the study set, and the population count ratio is the ratio of genes associated with the GO term to all genes in the population set.

GO ID	GO term	P value	Count ratio
GO:0010926	Anatomical structure formation	0.000	8.6/2.7
GO:0043473	Pigmentation	0.005	21.9/19.2
GO:0022610	Biological adhesion	0.007	2.6/1.7
GO:0065007	Biological regulation	0.000	23.7/20.1
GO:0044085	Cellular component biogenesis	0.000	5.8/2.2
GO:0016043	Cellular component organization	0.000	13.5/4.3
GO:0032502	Developmental process	0.028	18.5/6.8
GO:0040007	Growth	0.000	1.9/0.5
GO:0002376	Immune system process	0.000	4.3/1.1
GO:0050896	Response to stimulus	0.000	7.8/4.0

Gene pathway analysis

As previously described (Jiang *et al*., 2014), downstream pathway analysis in non-model fish species was conducted using KEGG pathway analysis combined with manual literature searches. In order to reveal the genetic basis underlying morphological traits of purse red carp, we mainly focused on skin color or body shape related pathways. We included 1) Melanin biosynthesis pathway, 2) TGF-beta signaling pathway, 3) Wnt signaling pathway, 4) MAPK signaling pathway.

Melanin biosynthesis pathway

Melanin is one of the main pigments present in vertebrates. Melanin biosynthesis is a complex pathway controlled by multiple factors, such as myriad hormonal factors and nutritional regulators, including melanocortins, histamines, eicosanoids, catecholamines, vitamin D, acetylcholine, androgens, dopamine, aromatic amino acid L-tyrosine and L-DOPA, and others (Slominski *et al*., 2004). It has been reported that the pigmentation process such as the development and differentiation of pigment cells, and the structure of tyrosinase, are largely conserved between mammals and teleost (Braasch *et al*., 2007; Slominski *et al*., 2004). As we previously demonstrated the putative gene pathways involved in the common carp skin pigmentation process (Jiang *et al*., 2014), in brief, melanocortins binds to the G-protein-coupled receptor MC1R, resulting the rising of cAMP levels, which triggers the eumelanin biosynthesis process. Amino acid tyrosine is first oxidized to dihydroxyphenylalanine (DOPA) by enzyme tyrosinase (TYR) and then to dopaquinone. Dopaquinone undergoes a series of reactions and rearrangements to form eumelanin, successively catalyzed by DCT, TYRP1 and SILV/PMEL. As shown in Table IV, melanin biosynthesis related genes identified in our study were significantly down-regulated in purse red carp skin. For instance, tyrosinase-related protein 1b (*Tyrp1b*) was 4.47-fold down-regulated in purse red carp, comparing with Yellow River carp, suggesting that the synthesis of eumelanin which contributes to brownish-black coloring is less active in red skin fish. *Tyrp1*, a key gene acting in eumelanin synthesis pathway, is expressed specifically in melanocytes. It was the first cloned pigmentation gene and later mapped to the *brown* locus in mouse (Jackson, 1988). *Tyrp1* has been reported that it plays a crucial role in pigmentation and color patterning in human (Alonso *et al*., 2008), mouse (Shibahara *et al*., 1992) and other mammals (Schmutz *et al*., 2002; Berryere *et al*., 2003; Schmidt-Kuntzel *et al*., 2005; Gratten *et al*., 2007), by affecting melanin synthesis, maintaining melanin structure, stabilizing tyrosinase protein, modulating tyrosinase catalytic activity, and affecting melanocyte proliferation and cell death (Rad *et al*., 2004; Kobayashi and Hearing, 2007). The essential role of *Tyrp1* in eumelanin formation has been reported in teleost fish as well (Braasch *et al*., 2009). Consistent with our previous study (Jiang *et al*., 2014), our results in the present study suggested an important role of *Tyrp1* in the skin coloration of the common carp.

Table IV.- The representative differentially expressed genes involved in the pigmentation- or body shape-related pathway.

Pathway involved	Gene name	Gene ID	Contig ID	Fold change
Melanin biosynthesis	TYRP1b	ENSDARG00000056151	Comp712271_c0_seq1	-4.47
	PMELa	ENSDARG00000091298	Comp584237_c0_seq1	-6.39
	MREG	ENSDARG00000011076	comp491208_c0_seq1	-5.77
	KITa	ENSDARG00000043317	Comp90489_c0_seq2	-8.04
TGF-beta signaling	BMP8a	ENSDARG00000035677	Comp380049_c0_seq1	6.28
	TGFB1a	ENSDARG00000041502	Comp101369_c0_seq1	6.25
	TGFB3	ENSDARG00000019367	Comp145484_c0_seq6	6.07
	TGFBR2	ENSDARG00000059363	Comp42703_c0_seq3	6.33
	ACVR2Aa	ENSDARG00000011188	Comp46038_c0_seq8	4.53
	TMX2b	ENSDARG00000007786	Comp24138_c0_seq1	7.47
	TBRG4	ENSDARG00000074807	Comp71491_c0_seq4	-6.64
	CUL1a	ENSDARG00000019239	Comp23712_c0_seq7	4.34
Wnt signaling	SFRP2	ENSDARG00000070050	Comp602807_c0_seq1	-2.95
	SFRP5	ENSDARG00000039041	Comp221054_c0_seq1	-4.89
	EP300b	ENSDARG00000061108	Comp39247_c0_seq3	7.17
	FZD7b	ENSDARG00000027589	Comp28378_c0_seq7	6.07
	CTBP1	ENSDARG00000019213	Comp11275_c0_seq28	-3.45
	NLK2	ENSDARG00000028793	Comp21019_c0_seq2	7.75
	DAAM1b	ENSDARG00000009689	Comp85163_c0_seq9	-6.54
	PLCB3	ENSDARG00000068246	Comp45746_c0_seq1	-2.94
	RAC3b	ENSDARG00000020795	Comp45162_c0_seq2	-6.98
	ROCK2a	ENSDARG00000017500	Comp43455_c0_seq3	-2.27
	GSK3aa	ENSDARG00000029501	Comp101890_c0_seq3	-5.83
	PPP3ca	ENSDARG00000004988	Comp31117_c0_seq1	-6.43
	PPARdb	ENSDARG00000009473	Comp67754_c0_seq15	4.57
	PRKCbb	ENSDARG00000022254	Comp57868_c0_seq1	7.91
MAPK signaling	FGFR1bl	ENSDARG00000075027	Comp44473_c0_seq1	-7.37
	FGFR2	ENSDARG00000058115	Comp69804_c0_seq2	-7.89
	FGFR4	ENSDARG00000069105	Comp853976_c0_seq1	-6.38
	FGFRl1a	ENSDARG00000032617	Comp483217_c0_seq1	-5.91
	FAS	ENSDARG00000043586	Comp21560_c0_seq1	9.13
	MAPK1	ENSDARG00000027552	Comp14373_c0_seq1	-8.05
	MAP3K2	ENSDARG00000062884	Comp62167_c0_seq6	-6.14
	MAP3K3	ENSDARG00000060348	Comp11068_c0_seq5	-6.79
	DUSP8a	ENSDARG00000009299	Comp118293_c0_seq1	-2.24
	ELK4	ENSDARG00000077092	Comp103271_c0_seq9	-9.08
	MAPK1	ENSDARG00000027552	Comp14373_c0_seq1	-8.05
	TAB2	ENSDARG00000021509	Comp19810_c0_seq1	7.24
	RAP1b	ENSDARG00000008867	Comp5345_c1_seq5	7.19
	JUND	ENSDARG00000067850	Comp32260_c0_seq14	-6.64
	MAPT	ENSDARG00000087616	Comp28341_c0_seq3	7.06
	NF1	ENSDARG00000012982	Comp101693_c0_seq2	7.70
	PLA2g4a	ENSDARG00000017141	Comp462446_c0_seq1	3.38
	PDGFB	ENSDARG00000038139	Comp122217_c0_seq2	-2.14

Other significant eumelanin synthesis related genes identified in this study included kit receptor a (*KITa/c-kit*), premelanosome protein a (*SILV/PMEL*), melanoregulin (*MREG*), which were significantly down-regulated in purse red carp as well, with 8.04-, 6.39-, and 5.71-fold changes, respectively (Table IV). *c-kit* gene encodes tyrosine kinase receptor. The expression of *c-kit* is not only essential for migration and survival of neural-crest-derived melanocyte precursors (Pielberg *et al*., 2002), but also for the transition of melanocytes to mature melanocytes (Hou *et al*., 2000). If *c-kit* is missing or nonfunctional, melanoblasts or at least their immediate precursor cells are still generated, but will be lost soon after their emergence from the crest (Hou *et al*., 2000). *PMEL* gene encodes premelanosome protein, which can catalyze the conversion of indole-5,6-quinone carboxylic acid into eumelanin (Chakraborty *et al*., 1996). Mutations of *PMEL* causing a reduction of black pigment have been reported in a number of vertebrate species (Brunberg *et al*., 2006; Karlsson *et al*., 2009), indicating critical roles of *PMEL* gene for normal melanosome development and epidermal pigmentation.

TGF-beta signaling pathway

The transforming growth factor beta (TGF-beta) signaling pathway is a metazoan-specific intercellular signaling pathway, which is involved in a wide range of developmental and cellular processes including cell growth, cell differentiation, apoptosis, and others. The TGF-beta superfamily involved in this pathway is comprised of dozens of structurally related polypeptides, which are divided into two major classes: the TGF-beta-like (TGF-β) subfamily and the bone morphogenetic protein-like (Bmp) subfamily. Those TGF-β proteins are synthesized as large precursor molecules which undergo proteolytic cleavage before released, then bind to a variety of single-pass transmembrane receptors, and transmit their signal through intracellular SMAD proteins, which in turn regulates many downstream target genes (Ahi, 2016) to function in a wide range of biological processes. Most of the members of TGF-β superfamily are ubiquitously expressed, and have been shown to play prominent roles in skeletal development and morphogenesis (Lin and Hankenson, 2011; Monroe *et al*., 2012), which consequently, could affect the body shape. In this study, our result showed that TGF-β1, TGF-β3 and TGF-β receptor II were significantly high-regulated in purse red carp (Table IV), with 6.25-, 6.07- and 6.33-fold change, respectively. Considering the special purse-like body shape of purse red carp with relatively high ratio of body depth to its body length, we speculated that TGF-βs and their receptors might be involved in the development and morphogenesis of bone in the dorsal-ventral axis of carp. Bone morphogenetic proteins (BMP) belongs to the TGF-β superfamily and function in many important developmental events including cartilage and bone formation, skeleton development, dorsoventral patterning, left-right asymmetry, organ formation (Reber-Muller *et al*., 2006). Bmp8, playing important role in bone metabolism (Kosa *et al*., 2011), was significantly highly expressed in purse red carp comparing with Yellow River carp, suggesting that Bmp8 might affect the body shape of purse red carp by regulating the bone formation. Our result showed that activin receptor IIA gene (*ACVR2A*) was expressed significantly higher in purse red carp. The activin receptors type II (A and B) is the cell surface receptor for multiple TGF-β superfamily ligands, which is responsible for the signal transduction to regulate skeletal muscle growth. Using transgenic technologies, Phelps *et al*. (2013) found that the transgenic rainbow trout with high levels expression of activin receptor type II B developed enhanced, localized musculature in both the epaxial and hypaxial regions, and examination of the interior abdominal wall of the transgenic fish revealed protrusions of muscle between the hemapophysial ribs corresponding to locations of external ridges. Their study provided compelling evidence for the importance of activin receptors in regulating muscle growth in teleost fish. Considering the high conservation of activin recetptor II A and B, we speculated that activin receptor II A has a similar function as activin receptor II B in regulating muscle growth. However, further studies are needed to verify its function.

Wnt and MAPK signaling pathway

As described in our previous study, both Wnt and MAPK signaling pathways are crucial for multiple developmental and physiological process, and they are highly likely to be involved in melanin biosynthesis in common carp (Jiang *et al*., 2014). Similarly, a group of genes involving in Wnt or MAPK signaling pathway were detected with significantly differentially expression level in purse red carp (Table IV). It is interesting that besides in the development of pigment cells, Wnt and MAPK signaling pathways also take part in morphogenesis. Wnt dependent signals are among the most studied molecular cascades in skeletal biology, due to their significant role in skeletogenesis (Monroe *et al*., 2012), and their modulatory function by interacting with other morphogenesis related molecules such as BMPs (Lin and Hankenson, 2011). MAPK signaling pathway could be activated and regulated by extracellular growth factor stimulation, then could function with FGF signaling pathway to establish a gradient of cell velocity enabling continuous rearrangement of the cells (Gros *et al*., 2010),

or regulating the segmentation process in embryo (Delfini *et al*., 2005). We speculated that the Wnt and MAPK signaling pathways could interact with other regulatory pathways such as melanogenesis process, morphogenesis process, immune system, or nervous system. However, the details of pathway interactions in purse red carp need to be further investigated.

CONCLUSIONS

In conclusion, we conducted a comparative transcriptomic analysis between two common carp varieties with distinct skin colors and body shapes to better understand the genetic mechanisms of morphological traits of purse red carp resulted from domestication. We detected 2767 unique genes that were significantly differentially expressed in the two carp varieties. Further annotation, GO enrichment and pathway analysis indicated that the candidate genes were mainly involved in at least four pathways that potentially affect the morphological traits of common carp. These results advanced our knowledge of the molecular mechanisms of skin color and body shape in teleost fish, and will facilitate the genetic selection and breeding of common carp with consumer-favored morphological traits.

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Statement of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

Ahi, E.P., 2016. Signalling pathways in trophic skeletal development and morphogenesis: Insights from studies on teleost fish. *Devel. Biol.,* **420**: 11-31. https://doi.org/10.1016/j.ydbio.2016.10.003

Alonso, S., Izagirre, N., Smith-Zubiaga, I., Gardeazabal, J., Diaz-Ramon, J.L., Diaz-Perez, J.L., Zelenika, D., Boyano, M.D., Smit, N. and de la Rua, C., 2008. Complex signatures of selection for the melanogenic loci TYR, TYRP1 and DCT in humans. *BMC Evol. Biol.,* **8**: 74. https://doi.org/10.1186/1471-2148-8-74

Balon, E., 1995. Origin and domestiction of the wild carp, *Cyprinus carpio*: from Roman gourmets to the swimming flowers. *Aquaculture,* **129**: 3-48. https://doi.org/10.1016/0044-8486(94)00227-F

Bar, I., Kaddar, E., Velan, A. and David, L., 2013. Melanocortin receptor 1 and black pigmentation in the Japanese ornamental carp (*Cyprinus carpio* var. Koi). *Front. Genet.,* **4**:6. https://doi.org/10.3389/fgene.2013.00006

Berryere, T.G., Schmutz, S.M., Schimpf, R.J., Cowan, C.M. and Potter, J., 2003. TYRP1 is associated with dun coat colour in Dexter cattle or how now brown cow? *Anim. Genet.,* **34**: 169-175. https://doi.org/10.1046/j.1365-2052.2003.00985.x

Braasch, I., Liedtke, D., Volff, J.N. and Schartl, M., 2009. Pigmentary function and evolution of tyrp1 gene duplicates in fish. *Pigment Cell Melanoma Res.,* **22**: 839-850. https://doi.org/10.1111/j.1755-148X.2009.00614.x

Braasch, I., Schartl, M. and Volff, J.N., 2007. Evolution of pigment synthesis pathways by gene and genome duplication in fish. *BMC Evol. Biol.,* **7**: 74. https://doi.org/10.1186/1471-2148-7-74

Brunberg, E., Andersson, L., Cothran, G., Sandberg, K., Mikko, S. and Lindgren, G., 2006. A missense mutation in PMEL17 is associated with the silver coat color in the horse. *BMC Genet*., **7**: 46. https://doi.org/10.1186/1471-2164-7-46

Chakraborty, A.K., Platt, J.T., Kim, K.K., Kwon, B.S., Bennett, D.C. and Pawelek, J.M., 1996. Polymerization of 5,6-dihydroxyindole-2-carboxylic acid to melanin by the pmel 17/silver locus protein. *Eur. J. Biochem.,* **236**: 180-188. https://doi.org/10.1111/j.1432-1033.1996.t01-1-00180.x

Chistiakov, D.A. and Voronova, N.V., 2009. Genetic evolution and diversity of common carp *Cyprinus carpio* L. *Cent. Eur. J. Biol.,* **4**: 304-312. https://doi.org/10.2478/s11535-009-0024-2

Delfini, M.C., Dubrulle, J., Malapert, P., Chal, J. and Pourquie, O., 2005. Control of the segmentation process by graded MAPK/ERK activation in the chick embryo. *Proc. natl. Acad. Sci. USA,* **102**: 11343-11348. https://doi.org/10.1073/pnas.0502933102

Fu, L., Niu, B., Zhu, Z., Wu, S. and Li, W., 2012. CD-HIT: Accelerated for clustering the next-generation sequencing data. *Bioinformatics,* **28**: 3150-3152. https://doi.org/10.1093/bioinformatics/bts565

Gratten, J., Beraldi, D., Lowder, B.V., McRae, A.F., Visscher, P.M., Pemberton, J.M. and Slate, J., 2007. Compelling evidence that a single nucleotide substitution in TYRP1 is responsible for coat-colour polymorphism in a free-living population of Soay sheep. *Proc. biol. Sci.,* **274**: 619-626. https://

doi.org/10.1098/rspb.2006.3762
Gros, J., Hu, J.K., Vinegoni, C., Feruglio, P.F., Weissleder, R. and Tabin, C.J., 2010. WNT5A/JNK and FGF/MAPK pathways regulate the cellular events shaping the vertebrate limb bud. *Curr. Biol*., **20**: 1993-2002. https://doi.org/10.1016/j.cub.2010.09.063
Hou, L., Panthier, J.J. and Arnheiter, H., 2000. Signaling and transcriptional regulation in the neural crest-derived melanocyte lineage: Interactions between KIT and MITF. *Development,* **127**: 5379-5389.
Jackson, I.J., 1988. A cDNA encoding tyrosinase-related protein maps to the brown locus in mouse. *Proc. natl. Acad. Sci. USA,* **85**: 4392-4396. https://doi.org/10.1073/pnas.85.12.4392
Jiang, Y., Zhang, S., Xu, J., Feng, J., Mahboob, S., Al-Ghanim, K.A., Sun, X. and Xu, P., 2014. Comparative transcriptome analysis reveals the genetic basis of skin color variation in common carp. *PLoS One*, **9**: e108200. https://doi.org/10.1371/journal.pone.0108200
Karlsson, A.C., Kerje, S., Hallbook, F. and Jensen, P., 2009. The Dominant white mutation in the PMEL17 gene does not cause visual impairment in chickens. *Vet. Ophthalmol.,* **12**: 292-298. https://doi.org/10.1111/j.1463-5224.2009.00714.x
Kobayashi, T. and Hearing, V.J., 2007. Direct interaction of tyrosinase with Tyrp1 to form heterodimeric complexes *in vivo*. *J. Cell Sci.,* **120**: 4261-4268. https://doi.org/10.1242/jcs.017913
Kosa, J.P., Kis, A., Bacsi, K., Balla, B., Nagy, Z., Takacs, I., Speer, G. and Lakatos, P., 2011. The protective role of bone morphogenetic protein-8 in the glucocorticoid-induced apoptosis on bone cells. *Bone*, **48**: 1052-1057. https://doi.org/10.1016/j.bone.2011.01.017
Langmead, B., Trapnell, C., Pop, M. and Salzberg, S.L., 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol*., **10**: R25. https://doi.org/10.1186/gb-2009-10-3-r25
Li, S.F., Wang, C.H. and Cheng, Q.Q., 2005. Morphological variations and phylogenesis of four strains in *Cyprinus carpio*. *J. Fish. China*, **29**: 606-611.
Lin, G.L. and Hankenson, K.D., 2011. Integration of BMP, Wnt, and notch signaling pathways in osteoblast differentiation. *J. cell. Biochem*., **112**: 3491-3501. https://doi.org/10.1002/jcb.23287
Monroe, D.G., McGee-Lawrence, M.E., Oursler, M.J. and Westendorf, J.J., 2012. Update on Wnt signaling in bone cell biology and bone disease. *Gene*, **492**: 1-18. https://doi.org/10.1016/j.gene.2011.10.044
Phelps, M.P., Jaffe, I.M. and Bradley, T.M., 2013. Muscle growth in teleost fish is regulated by factors utilizing the activin II B receptor. *J. exp. Biol.,* **216**: 3742-3750. https://doi.org/10.1242/jeb.086660
Pielberg, G., Olsson, C., Syvanen, A.C. and Andersson, L., 2002. Unexpectedly high allelic diversity at the KIT locus causing dominant white color in the domestic pig. *Genetics*, **160**: 305-311.
Rad, H.H., Yamashita, T., Jin, H.Y., Hirosaki, K., Wakamatsu, K., Ito, S. and Jimbow, K., 2004. Tyrosinase-related proteins suppress tyrosinase-mediated cell death of melanocytes and melanoma cells. *Exp. Cell Res.,* **298**: 317-328. https://doi.org/10.1016/j.yexcr.2004.04.045
Reber-Muller, S., Streitwolf-Engel, R., Yanze, N., Schmid, V., Stierwald, M., Erb, M. and Seipel, K., 2006. BMP2/4 and BMP5-8 in jellyfish development and transdifferentiation. *Int. J. Devel. Biol.,* **50**: 377-384. https://doi.org/10.1387/ijdb.052085sr
Robinson, M.D., McCarthy, D.J. and Smyth, G.K., 2010. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, **26**: 139-140. https://doi.org/10.1093/bioinformatics/btp616
Schmidt-Kuntzel, A., Eizirik, E., O'Brien, S.J. and Menotti-Raymond, M., 2005. Tyrosinase and tyrosinase related protein 1 alleles specify domestic cat coat color phenotypes of the albino and brown loci. *J. Hered.,* **96**: 289-301. https://doi.org/10.1093/jhered/esi066
Schmutz, S.M., Berryere, T.G. and Goldfinch, A.D., 2002. TYRP1 and MC1R genotypes and their effects on coat color in dogs. *Mammal. Genome,* **13**: 380-387. https://doi.org/10.1007/s00335-001-2147-2
Shibahara, S., Tomita, Y., Yoshizawa, M., Shibata, K. and Tagami, H., 1992. Identification of mutations in the pigment cell-specific gene located at the brown locus in mouse. *Pigment Cell Res. Suppl.,* **2**: 90-95.
Slominski, A., Tobin, D.J., Shibahara, S. and Wortsman, J., 2004. Melanin pigmentation in mammalian skin and its hormonal regulation. *Physiol. Rev.,* **84**: 1155-1228. https://doi.org/10.1152/physrev.00044.2003
Wang, C., Wachholtz, M., Wang, J., Liao, X. and Lu, G., 2014. Analysis of the skin transcriptome in two oujiang color varieties of common carp. *PLoS One,* **9**: e90074. https://doi.org/10.1371/journal.pone.0090074
Wang, C.H., Li, S.F., Nagy, Z.T., Lehoczky, I., Huang, L., Zhao, Y., Song, X. and Jeney, Z., 2010. Molecular

genetic structure and relationship of Chinese and Hungarian common carp (*Cyprinus carpio* L.) strains based on mitochondrial sequence. *Aquacul. Res.,* **41**: 1339-1347. https://doi.org/10.1111/j.1365-2109.2009.02422.x

Xu, L.H., Wang, C.H., Wang, J., Dong, Z.J., Ma, Y.Q. and Yang, X.X., 2012. Selection pressures have driven population differentiation of domesticated and wild common carp (*Cyprinus carpio* L.). *Genet. Mol. Res.,* **11**: 3222-3235. https://doi.org/10.4238/2012.September.12.5

Ye, J., Fang, L., Zheng, H., Zhang, Y., Chen, J., Zhang, Z., Wang, J., Li, S., Li, R. and Bolund, L., 2006. WEGO: A web tool for plotting GO annotations. *Nucl. Acids Res.,* **34**: W293-297. https://doi.org/10.1093/nar/gkl031

Zdobnov, E.M. and Apweiler, R., 2001. InterProScan--an integration platform for the signature-recognition methods in InterPro. *Bioinformatics,* **17**: 847-848. https://doi.org/10.1093/bioinformatics/17.9.847

Zhang, Y., Wang, S., Li, J., Zhang, X., Jiang, L., Xu, P., Lu, C., Wan, Y. and Sun, X., 2013. Primary genome scan for complex body shape-related traits in the common carp *Cyprinus carpio*. *J. Fish Biol*., **82**: 125-140. https://doi.org/10.1111/j.1095-8649.2012.03469.x