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Solute carriers (SLCs) identified and characterized from kidney transcriptome of golden mahseer (*Tor putitora*) (Fam: Cyprinidae)

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ABSTRACT

The solute carriers (SLC) are trans-membrane proteins, those regulate the transport of various substances (sugars, amino acids, nucleotides, inorganic cations/anions, metals, drugs etc.) across the cell membrane. There are more than 338 solute carriers (slc) reported in fishes that play crucial role in cellular influx and efflux. The study of solute carrier families may reveal many answers regarding the function of transporter genes in the species and their effect in the existing environment. Therefore, we performed RNA sequencing of kidney tissue of the golden mahseer (*Tor putitora*) using Illumina platform to identify the solute carrier families and characterized 24 putative functional genes under 15 solute carrier families. Out of 24 putative functional genes, 11 genes were differentially expressed in different tissues (head kidney, trunk kidney, spleen, liver, gill, muscle, intestine and brain) using qRT-PCR assay. The slc5a1, slc5a12, slc12a3, slc13a3, slc22a13 and slc26a6 were highly expressed in kidney. The slc15a2, slc25a47, slc33a1 and slc38a2 were highly expressed in brain and slc30a5 was over-expressed in gill. The unrooted phylogenetic trees of slc2, slc5, slc13 and slc33 were constructed using amino acid sequences of *Homo sapiens*, *Salmo salar*, *Danio rerio*, *Cyprinus carpio* and *Tor putitora*. It appears that all the putative solute carrier families are very much conserved in human and fish species including the present fish, golden mahseer. This study provides the first hand database of solute carrier families particularly transporter encoding proteins in the species.

1. Introduction

The solute carriers are very important group of genes that play a major role in membrane transport and transport regulation of various substances (sugars, amino acids, nucleotides, inorganic cations/anions, metals, drugs etc.) across the membrane. The solute carriers control the uptake and efflux of essential cellular compounds, environmental toxins, therapeutic drugs, etc. and even considered as a potential target for cancer treatment (El-Gebali et al., 2013). The importance of understanding the functional diversity and expression patterns of solute carriers is reflected by the number of recent reviews on mammals and other vertebrates (Hediger et al., 2004; Nigam et al., 2007; Vasiliou et al., 2009: Verri et al., 2012; Pajor, 2014) and a special volume published by Pflugers Archiv-Europian Journal of Physiology (PAEJP, 2004).

The Gene Nomenclature Committee (HGNC) of the Human Genome Organization (HUGO) had enlisted 396 putative solute carrier (SLC) genes in humans (Povey et al., 2001). These genes were further grouped among 52 solute carrier families based on their sequence similarity (minimum 20%) and substrate relatedness with other members of the family (Hediger et al., 2004). After the advent of next generation sequencing technology, gene discovery has reached to its peak and consequently genomic resources particularly on genes coding for transporters are obtained in many mammals and vertebrates. The isolation and characterization of solute carrier families and their members were also reported in several species under teleost group of fishes (Bury et al., 2008; Verri et al., 2012; Hsu et al., 2014). There are 50 families of solute carrier representing 338 putatively functional protein coding genes in teleost fishes and 304 slcs among them were observed in zebrafish (*Danio rerio*) alone (Verri et al., 2012). The zebrafish has been adopted as model organism to validate the transporter encoding gene expression analysis by several authors (Bayaa et al., 2009; Ho et al., 2012; Tian et al., 2015).

The golden mahseer (*Tor putitora*) is a Himalayan fish species which is declining in several streams and rivers due to many abiotic and biotic pressures. The species has been tagged as endangered in IUCN red list of threatened species (http://www.iucnredlist.org). It is also reported that the abundance of this species may reduce to 80% in the future (Jha and Rayamajhi, 2010). The study of solute carrier families may reveal many answers regarding the role of transporter encoding genes and their functional role in the existing environment. As far as we are aware after intensive literature survey, there is no such information available

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Table 1
The details of solute carrier families observed in kidney transcriptome of *Tor putitora* (classification of SLC family was followed according to Verri et al., 2012; He et al., 2009).

Transcript ID	Solute carrier family	Members found	Official symbol	Gene/other designations	Species of Blastx similarity	Gene acc.
1. Inorganic cation/anion transport						
21,067	slc4	2a	slc4a2	Anion exchange protein 2	*Danio rerio*	NP_001032314
21,068	slc4	2	slc4a2	Anion exchanger 2	*Danio rerio*	NP_001032314
11,893	slc8	2	slc8a2	Sodium-calcium exchanger 2 precursor	*Danio rerio*	NP_001116768
603	slc12	3	slc12a3	Sodium/chloride transporters	*Danio rerio*	NP_001038545
14,657	slc26	6	slc26a6	Anion exchanger; sulphate transporter N-terminal domain with GLY motif	*Danio rerio*	NP_001107889
2. Amino acid and oligopeptide transport						
5363	slc15	2	slc15a2	H+/peptide transporter	*Danio rerio*	NP_001034917
13,674	slc 38	2	slc38a2	Sodium-coupled neutral amino acid transporter A2	*Danio rerio*	NP_001038569
3. Transport of glucose and other sugars						
159	slc2	11b	slc2a11b	Glucose transporter; The Major Facilitator Superfamily (MFS)	*Danio rerio*	NP_001107902
3573	slc2	12	slc2a12	Glucose transporter; GLUT-12	*Danio rerio*	NP_956832
2257	slc5	1	slc5a1	Na+/glucose cotransporter	*Cyprinus carpio*	AEX13746
6590	slc5	11	slc5a11	Sodium/myo-inositol co-transporter	*Danio rerio*	NP_001007301
13,394	slc5	2	slc5a2	Sodium/glucose cotransporter 2	*Danio rerio*	NP_998091
9147	slc 5	12	slc5a12	Sodium-coupled mono carboxylate transporter 2	*Danio rerio*	NP_956662
4. Transport of bile salts and organic anions						
10,935	slc13	1	slc13a1	Sodium/sulphate symporters	*Danio rerio*	AAH66761
10,937	slc13	1	slc13a1	Sodium/sulphate symporters	*Danio rerio*	AAH66761
4169	slc13	3	slc13a3	Di- and tricarboxylate transporter	*Danio rerio*	NP_998067
15,288	slc13a	5	slc13a5	Sodium-dependent citrate transporter	*Danio rerio*	AEF30425
5. Metal ion transport						
11,854	slc30	5	slc30a5	Zinc transporter 5	*Danio rerio*	NP_001002322
14,394	slc30	1	slc30a1	Zinc transporter 1; Co/Zn/Cd efflux system component [Inorganic ion transport and metabolism]	*Danio rerio*	NP_957173
6. Transport of urea, neurotransmitters and biogenic amines, ammonium and choline						
8	slc6	19b	slc6a19b	Sodium-dependent neutral amino acid transporter B(0)AT1	*Danio rerio*	NP_956030.1|
8226	slc22	13	slc22a13	Cation transport protein	*Danio rerio*	NP_001070840
2883	slc22	5	slc22a5	Organic cation/ergothioneine transporter	*Danio rerio*	NP_957143
7. Transport of vitamins and co-factors						
18,672	slc33	1	slc33a1	Acetyl-CoA transporter 1; major facilitator super family	*Danio rerio*	NP_957402
8. Transport across mitochondrial membranes						
268	slc25	47a	slc25a47a	Mitochondrial carrier protein; hepatocellular carcinoma down-regulated mitochondrial carrier homolog A	*Danio rerio*	NP_001038779

on solute carrier in this species. Therefore, the present study was undertaken to generate first hand genomic information regarding the solute carriers from kidney transcriptome of golden mahseer using next generation sequencing platform. This database may be useful to study the genome evolution and gene-environment interaction with particular reference to transporter encoding proteins.

2. Material and methods

2.1. Sample collection

Live samples of the golden mahseer (*Tor putitora*) were collected from the Kosi River (Ramnagar, 29.40° N 79.12 ° E) and different organs were dissected out from one anesthetized fish. The size and weight of the fish was 15 cm and 75 g, respectively. The sex of the fish was unidentified. The kidney tissue was stored in RNAlater (Ambion Inc., Austin, Texas) at the sampling site and stored at −80 °C in the laboratory. All protocols were performed in accordance with Animal Welfare Act approved by ICAR-DCFR Ethical committee.

2.2. RNA isolation, library preparation and sequence run

Total RNA was isolated from kidney tissue (as well as other organs for qRT-PCR analysis) using TRIzol® reagent (Ambion Inc., Austin, Texas) and further purified using GeneJET RNA purification kit (Thermo Fisher Scientific, Wilmington, USA) as per manufacturer's recommendation. RNA was treated with DNAse I (Thermo Fisher Scientific, Wilmington, USA) to remove genomic DNA contamination. The concentration of total RNA was determined using Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, USA) and the quality of total RNA was checked on 1.2% denatured agarose gel. The cDNA library preparation was carried out using TruSeq RNA Library Preparation Kit v.2 (Illumina, San Diego, USA) as per manufacturer's recommendation. The library preparation and sequencing was performed on Illumina Miseq 500 platform using 2 × 150 PE chemistry at Xcelris Genomics Ltd., Ahmedabad, India.

2.3. RNA sequence analysis, de novo assembly and annotation

The raw reads generated from Illumina sequencing was subjected to initial quality check using Fastx toolkit and CLC Genomics Workbench v.7.5.2 (CLC Bio, Aarhus, Denmark) followed by trimming for ambiguity, low quality and PCR duplicates. A *denovo* assembly of the cleaned reads was carried out using CLC Genomics Workbench v.7.5.2 with a minimum contig length of 200 bp and a trimming quality score of 0.05. The contigs were further assembled into unigenes with the help of sequence clustering software CAP3 (Huang and Madan, 1999). The assembled contigs and unigenes were used to identify the coding sequences using TransDecoder (http://transdecoder.github.io) on default parameters. Further, the coding regions (CDS) generated through Transdecoder were used for functional annotations using non-redundant database of Blastx of NCBI with an e-value threshold of 1e-6. From the Blastx results, the transcripts matched exclusively with solute carrier families were