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Molecular cloning and characterization of taurine transporter from turbot (*Psetta maxima*) and its expression analysis regulated by taurine *in vitro*

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Abstract

Taurine is one of the conditionally essential amino acids for fish. Its metabolism has attracted much attention in fish nutrition due to the substitution fishmeal with plant ingredients, which barely contain taurine. However, little is known about the mechanisms of taurine homoeostasis in turbot (*Psetta maxima*), an economically valuable carnivorous fish with limited taurine biosynthesis ability. In this study, we obtained the full-length cDNA of turbot taurine transporter (*Pm*TauT) and classified its tissue distribution in juvenile turbot and substrate regulation in isolated turbot muscle cells. Full-length *Pm*TauT cDNA was 2566 bp containing a 58 bp 5′ untranslated region (UTR), a 633 bp 3′UTR and a 1875 bp open reading frame (ORF) encoding 624 amino acid residues. The deduced protein contained 12 putative transmembrane domains and showed highest similarity with taurine transporter from Senegalese sole (*Solea senegalensis*), another flatfish species. By quantitative real-time PCR analysis, *Pm*TauT transcript was predominantly detected in liver and intestine, while was lesser expressed in gill and stomach. In addition, *Pm*TauT in isolated turbot muscles was down-regulated by taurine in a dose-dependent manner. These results suggested that *Pm*TauT was involved in taurine homoeostasis in turbot.

Keywords: *Psetta maxima*, taurine transporter, tissue expression, substrate regulation

Introduction

Taurine (2-aminoethane sulphonic acid) is one of the most abundant nonpeptidic amino acid in animal tissues (Schuller-Levis & Park 2003; Bouckenooghe, Remacle & Reusens 2006). It has been considered as an essential nutrient for many fish species due to their limited taurine biosynthesis abilities (Takagi, Murata, Goto, Endo, Yamashita & Ukawa 2008; Li, Mai, Trushenski & Wu 2009; Espe & Holen 2013; Lim, Oh, Khosravi, Cha, Park, Kim & Lee 2013; Salze & Davis 2015). Taurine has multiple physiological functions in fish, such as growth stimulation (Lunger, McLean, Gaylord, Kuhn & Craig 2007), osmoregulation (Takagi, Murata, Goto, Hayashi, Hatate, Endo, Yamashita & Ukawa 2006a; Kato, Yamamoto, Peerapon, Fukada, Biswas, Yamamoto, Takii & Miyashita 2014), bile salt conjugation (Kim, Matsunari, Takeuchi, Yokoyama, Murata & Ishihara 2007), antioxidant defence (Espe & Holen 2013) and larvae development (Pinto, Figueira, Ribeiro, Yúfera, Dinis & Aragão 2010).

During the past decades, taurine metabolism has attracted much attention in fish nutrition studies due to the fact that plant ingredients replacement of fishmeal in aquafeeds (Li *et al.* 2009; Kuzmina, Gavrovskaya & Ryzhova 2010; El-Sayed 2014; Salze & Davis 2015). The sources of taurine in fish are *de novo* synthesis and dietary. However, many fish species have extremely low taurine biosynthesis abilities and they are much more dependent on dietary intake (Yokoyama, Takeuchi, Park & Nakazoe 2001; Goto, Matsumoto, Murakami, Takagi & Hasumi 2003; Kim, Matsunari, Takeuchi, Yokoyama, Furuita, Murata & Goto 2008). Fishmeal is known to be the major source of taurine in aquafeeds. Due to the high price and limited supply, numerous studies on replacing fish meal with plant ingredients have been carried out, which usually resulted in a

deficiency of taurine in diets (Tacon & Metian 2008). Many defects associated with taurine deficiency have been observed in fish, such as growth depression (Lunger *et al.* 2007; Chatzifotis, Polemitou, Divanach & Antonopoulou 2008; Al-Feky, El-Sayed & Ezzat 2015), poor disease resistance (Maita, Maekawa, Satoh, Futami & Satoh 2006), oxidative stress (Espe, Ruohonen & El-Mowafi 2012) and high mortality (Salze, Craig, Smith, Smith & McLean 2011). Supplementation of taurine to plant protein diets has been proved indispensable to sustain comparable levels of feed intake and fish growth (Lunger *et al.* 2007; Al-Feky *et al.* 2015). It is important to further clarify the mechanism of taurine absorption and its homoeostasis in fish.

The absorption of dietary taurine and its homoeostasis in organisms were mainly regulated by taurine transporter (SLC6A6; TauT), a high-affinity, low-capacity sodium and chloride dependent transporter (O'Flaherty, Stapleton, Redmond & Bouchier-Hayes 1997). It transports taurine against a concentration gradient, driven by transmembrane sodium and chloride gradients and membrane potentials. TauT has been cloned and characterized in several fish species, including zebrafish (Kozlowski, Chen, Zhuang, Fei, Navarre & Ganapathy 2008), carp (Takeuchi, Toyohara & Sakaguchi 2000b), Atlantic salmon (Zarate & Bradley 2007), tilapia (Takeuchi, Toyohara, Kinoshita & Sakaguchi 2000a), Japanese eel (Chow, Ching, Wong & Wong 2009) and Senegalese sole (Pinto, Rønnestad, Jordal, Gomes, Dinis & Aragão 2012). These studies have demonstrated that TauT was important for maintenance the taurine body pool and its significant role of TauT in osmoregulation. However, the regulation of fish TauT by diet, which is critical for understanding taurine homoeostasis in fish and defining taurine supplementation in aquafeeds, was rarely addressed. Previously studies has shown that taurine transport capacity of the renal tubular cell was down-regulated by the dietary intake of taurine in mammals (Rozen & Scriver 1982; Chesney, Gusowski & Dabbagh 1985) and the feedback regulation of TauT by taurine was through modifying the transport maximal velocity (Han, Patters, Jones, Zelikovic & Chesney 2006). It is important to explore the substrate regulation of fish TauT.

Turbot *Psetta maxima* is an economically valuable carnivorous fish widely cultured in Europe and Asia. Previous studies have suggested that turbot has limited taurine biosynthesis ability (Conceição, van der Meeren, Verreth, Evjen, Houlihan & Fyhn 1997; Qi, Ai, Mai, Xu, Liufu, Yun & Zhou 2012; Yun, Ai, Mai, Xu, Qi & Luo 2012; Wang, He, Wang, Mai, Xu & Zhou 2014), while the homoeostasis of taurine in turbot remains unexplored. In this study, we obtained the full-length cDNA of turbot *P. maxima* taurine transporter (denoted as *Pm*TauT). The tissue distribution of *Pm*TauT in juvenile turbot and its feedback regulation by taurine in primary cultured turbot muscle cells were also investigated. These results will provide new insights into taurine homoeostasis in teleost.

Materials and methods

Sample collection

The juvenile turbot *P. maxima* (30–57 g in mass) was collected from a commercial farm in Qingdao (Shandong, China). Seawater was pumped from the coast adjacent to the farm and filtered with sand filters. During rearing at the farm, water temperature was ranged from 18.0 to 22.0°C; pH from 7.4 to 8.2; salinity from 26‰ to 30‰; ammonia nitrogen was lower than 0.1 mg L^{-1}; nitrite was lower than 0.1 mg L^{-1}; dissolved oxygen was higher than 5.0 mg L^{-1}. Fish was fed with commercial diet (Great Seven Bio-Tech, 49.5% protein, 10.2% lipid, 1.08% taurine) twice everyday. Before sampling, fish was fasted overnight to clear dietary influences (Kaushik & Luquet 1977). Fish was euthanized with 3-aminobenzoic acid ethyl ester (MS222) at 100 μg mL^{-1}. Samples of liver, muscle, stomach, intestine, heart, gill, brain and eye were rapidly removed and immediately frozen in liquid nitrogen. The samples were stored at −80°C until use. All procedures performed in study were in strict accordance with the ethical standards of the Helsinki declaration as revised in 1975.

RNA extraction and cDNA cloning

The full-length cDNA cloning of *Pm*TauT was carried out as described previously (Sambrook & Russell David 1989). Total RNA was extracted from tissues using Trizol RNA isolation reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The quality and concentration of RNA were measured by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

First strand cDNA was produced by SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, CA, USA) and used as template for 3′ or 5′ rapid amplification of cDNA ends (RACE) PCR. To obtain the full-length cDNA of *Pm*TauT, degenerative primers were designed on the conserved regions from different species using CODEHOP online software firstly. Subsequently, specific primers were further designed for RACE PCR. The RACE PCR amplification was conducted with a thermal cycling according to the manufacturer's instructions. All PCR products were separated by agarose gel electrophoresis and the expected-size DNA fragments were obtained, gel purified and then ligated into pEASY-T1 vector (TransGen, Beijing, China). After transformed into the competent cells of *E. coli* DH5α, the positive recombinants were identified through ampicillin selection and PCR screening with M13-F and M13-R. The positive clones were sequenced by Sangon Biotech (Shanghai, China). The full-length cDNA sequence of *Pm*TauT was deposited to the GenBank database (GenBank accession no. KT369001). All primers used in this study were listed in Table 1.

Sequence and phylogenetic analysis

The *Pm*TauT sequence was analysed using the National Center for Biotechnology Information BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast). The deduced amino acid sequence was analysed with the Expert Protein Analysis System (http://www.expasy.org). The putative transmembrane domains were predicted using TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM). Potential glycosylation sites were predicted by NetNGlyc 1.0 Server (http://www.cbs.dtu.dk/services/NetNGlyc).

Multiple sequence alignments of the deduced amino acid sequence of *Pm*TauT was conducted using the ClustalW software (http://www.ebi.ac.uk/tools/msa/clustalW2). Phylogenetic analysis was performed with MEGA 4.0. The phylogenetic tree was constructed on the basis of amino acid sequences using the neighbour-joining (NJ) method (Saitou & Nei 1987). Confidence in the resulting phylogenetic tree branch topology was measured by bootstrap analysis with 1000 replicates (Felsenstein 1985; Hedges 1992).

Tissue-specific expression of *Pm*TauT mRNA

The mRNA expression of *Pm*TauT in different tissues of juvenile turbot was measured by quantitative real-time PCR (qRT-PCR). Total RNA was extracted from liver, muscle, stomach, intestine, heart, gill, brain and eye. First strand cDNA was synthesized using the PrimeScript® RT Reagent Kit (Takara, Dalian, China). The qRT-PCR was carried out using the SYBR Green real-time PCR kit (Takara) with a quantitative thermal cycler CF96 (Bio-Rad, Foster City, CA, USA). GAPDH was used as the reference gene for internal standardization. The PCR program was as follows: (1) 15 s at 95°C for 1 cycle and (2) 10 s at 95°C, 10 s at 60°C and 20 s at 72°C for 40 cycles. The melting curve analysis of the amplification products was performed to demonstrate the specificity of the PCR products. Six samples of each tissue were analysis and each sample was run in triplicate ($n = 6$). Target gene expression levels were quantitated by the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen 2001). The *Pm*TauT expression levels of Gill were normalized to 1.0 and the rest tissues were expressed as relative expression values to the Gi group.

Table 1 Sequences of the primers used in this study

Name	Primer sequence (5′ – 3′)	Description
TauT-F	CTKCTGCTDYTGGGCCTCGACAG	Degenerate PCR
TauT-R	GGCTTNHTCAAGCCHYYGTTCC	Degenerate PCR
TauT-race F1	ACGAAAGGCGGCATGTATGT	3′ RACE PCR
TauT-race F2	TACAATGTGAAGGGCGAGTT	3′ RACE PCR
TauT-race R1	ATGGCGAGTCCCAGGAGATA	5′ RACE PCR
TauT-race R2	GTCCCTGTAGCAGTTGTATTTG	5′ RACE PCR
TauT-rt F	GGGATCGAGGACATAGGG	Real-time PCR
TauT-rt R	GTAGGCGGGTCAGGTTAG	Real-time PCR
GAPDH-rt F	AGCAGCAGCCATGTCAGACC	Real-time PCR
GAPDH-rt R	TTGGGAGACCTCACCGTTGTAAC	Real-time PCR
M13-F	TGTAAAACGACGGCCAGT	Universal primer
M13-R	CAGGAAACAGCTATGACC	Universal primer

Isolation and culture of turbot muscle cells

Fish (about 15 g) was obtained from a commercial farm in Qingdao (Shandong, China) and maintained in a tank (60 cm × 60 cm × 60 cm, about 20 fish per tank) connected with a closed-water flow circuit for a week. During the maintaining period, fish was fed with a commercial diet (Great-Seven Bio-Tech, 2# feed for turbot, 49.5% protein, 10.2% lipid, 1.08% taurine) and water temperature was ranged from 19.5 to 20.5°C; pH from 7.3 to 8.0; salinity from 30‰ to 31‰; ammonia nitrogen was lower than 0.1 mg L^{-1}; nitrite was lower than 0.1 mg L^{-1}; dissolved oxygen was higher than 5.0 mg L^{-1}. Before the sampling, fish was fasted for 24 h and sacrificed by a blow to the head and the skin was sterilized by immersion in 70% ethanol for 30 s. Turbot muscle cells were isolated according to the published protocols with slight modification (Castillo, Le Bail, Paboeuf, Navarro, Weil, Fauconneau & Gutierrez 2002). White dorsal muscle was excised under sterile conditions and collected in cold L-15 medium (Sigma, St. Louis, MO, USA) with antibiotics (penicillin 400 U mL^{-1}, streptomycin 400 μg mL^{-1}; Gibco, Gaithersburg, MD, USA). The tissue was minced and the fragments were centrifuged (300 g, 10 min at 4°C) and collected. Enzymatic digestion was performed with 0.05% Trypsin at room temperature on a shaker for 30 min. The reaction was neutralized with L-15 medium containing 10% foetal bovine serum (FBS, #10099141, taurine 237.4 ± 31.2 μM; Gibco). The cell suspension was purified through a cell strainer of 70-μm mesh size (BD Falcon, San Jose, CA, USA). The isolated muscle cells were collected in 15-mL sterilized centrifuge tubes and centrifuged (300 g, 10 min at 4°C). The cell pellets were resuspended with cold L-15 medium supplemented with 10% FBS, 20 mM HEPES, 1% antibiotics (penicillin 100 U mL^{-1}, streptomycin 100 μg mL^{-1}), 2 mM GlutaMAX™ and basic fibroblast growth factor (2.5 ng mL^{-1}; Gibco). Cells were cultured in 6-well plastic plates (Corning, New York, NY, USA) at 24°C in a normal atmosphere incubator. Observations and morphology were regularly made to control the state of the cells.

Substrate regulation of *Pm*TauT *in vitro*

In order to explore the effect of taurine on the expression of *Pm*TauT transcript, this study was carried out in turbot muscle cells adapted to a taurine-free chemically defined medium (Jayanthi, Ramamoorthy, Mahesh, Leibach & Ganapathy 1995; Bitoun & Tappaz 2000). Briefly, the cells were seeded in 6-well plastic plates at a density of 2×10^5 cells/well and allowed to grow for 24 h in the complete growth medium (10% FBS). Following this, the cells were adapted to a medium containing 5% FBS for 24 h and finally incubated for an additional 24 h in a chemically defined medium in the absence of serum (L-15 medium containing 20 mM HEPES, 1% antibiotics, 2 mM GlutaMAX™ and 2.5 ng mL^{-1} bFGF). And then, the cells were treated with experimental medium which contained various concentrations of taurine (0, 10, 25, 50, 100, 500 μM, respectively), which cover the physiological content in turbot muscle (Qi *et al.* 2012; Wang *et al.* 2014). The treated cells were harvested after 24 h to analyse the expression of *Pm*TauT transcript. All cell culture experiments were repeated for four times ($n = 4$). The *Pm*TauT expression levels of taurine-free group were normalized to 1.0 and the rest tissues were expressed as relative expression values to it.

Statistical analysis

The tissue distribution of *Pm*TauT was analysed using one-way ANOVA by SPSS 19.0 (SPSS, Chicago, IL, USA). Prior to the statistical tests, data were examined for homogeneity of variances (*F*-test). As the data were heterogeneous, we analysed them after log-transformed. Differences between the means were tested by Tukey's multiple range tests. The level of significance was chosen at $P < 0.05$ and the results were presented as means ± SEM (standard error of the mean). The result of substrate regulation of *Pm*TauT was estimated by broken-quadratic regression analysis.

Results

cDNA cloning and sequencing characterization of *Pm*TauT

The complete nucleotide and deduced amino acid sequence of *Pm*TauT were shown in Fig. 1. The full-length cDNA of *Pm*TauT was 2566 bp, containing a 5′ untranslated region (UTR) of 58 bp, a 3′ UTR of 633 bp and an open reading frame (ORF) of 1875 bp encoding 624 amino acids. The predicted molecular mass of *Pm*TauT was 69.7 kDa. The deduced protein

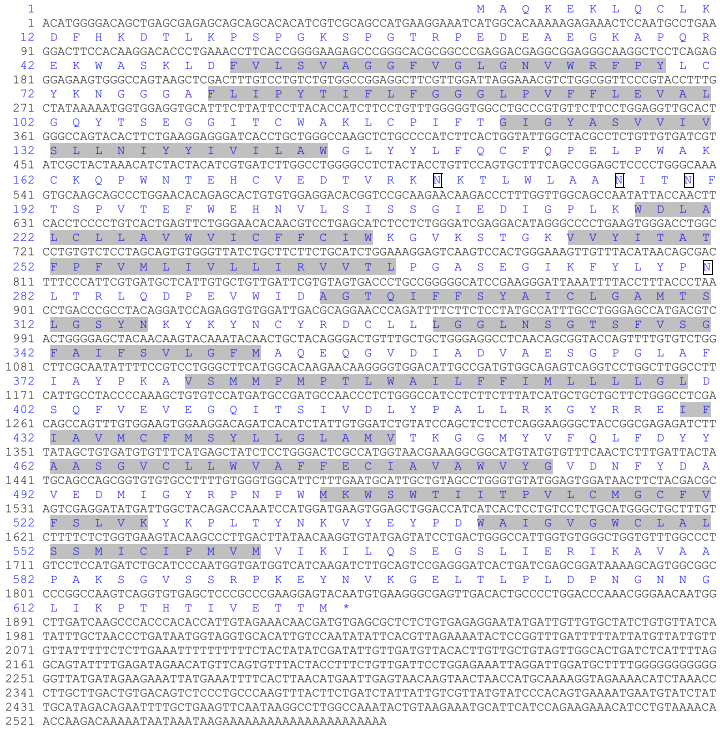

Figure 1 The full-length cDNA sequence and deduced amino acid sequence of *Pm*TauT. The deduced amino acid sequence was shown upon the nucleotide sequence. The asterisk indicated the stop codon. Nucleotides were numbered from the first base at the 5′ end. Four potential N-glycosylation sites were boxed. Twelve transmembrane domains were shadowed.

contained 12 putative transmembrane domains. In addition, four potential N-glycosylation sites were identified in deduced amino acid sequence of *Pm*TauT.

Homology and phylogenetic analyses of *Pm*TauT

The multiple amino acid sequence alignment revealed that taurine transporter showed high homology across species, while the C-terminus of the protein contained a more variable region (Fig. 2). Taurine transporter from turbot shared 93% identity with *Solea senegalensis*, no less than 89% with other teleost and at least 77% identity with mammals. Moreover, a Cysteine was found in amino acid residue 322 of *Pm*TauT while it was serine in mammals, which was a phosphorylation site of protein kinase C that controlled TauT activity.

A phylogenetic tree was constructed to evaluate the molecular evolutionary relationships of *Pm*TauT against other TauT proteins (Fig. 3). Based on the phylogenetic analysis, the TauTs from invertebrates and vertebrates were classified into two distinct clades. *Pm*TauT was most closely related to sequences from *Solea senegalensis* and *Salmo salar* with high bootstrap support. TauTs from mammals fell into two subclusters with other vertebrates that were in accordance with the results of homology analysis. The phylogenetic tree indicated that the evolution of TauT was almost in accord with the evolution of species.

Tissue-specific expression of *Pm*TauT

The real-time PCR assay showed that *Pm*TauT was ubiquitously observed in all examined tissues, including brain, eye, gill, heart, intestine, liver, muscle, and stomach (Fig. 4). The highest expression levels of *Pm*TauT were found in liver and intestine. The expression levels of *Pm*TauT transcript in gill and stomach were lower than that in other tissues.

Substrate regulation of *Pm*TauT expression in turbot muscle cells

Broken-quadratic regression analysis of the mRNA levels of *Pm*TauT was shown in Fig. 5. The expression levels of *Pm*TauT showed a dose-dependent decrease when taurine concentration was below 88.75 μM. The mRNA levels of *Pm*TauT were almost constant when taurine concentration was over 88.75 μM.

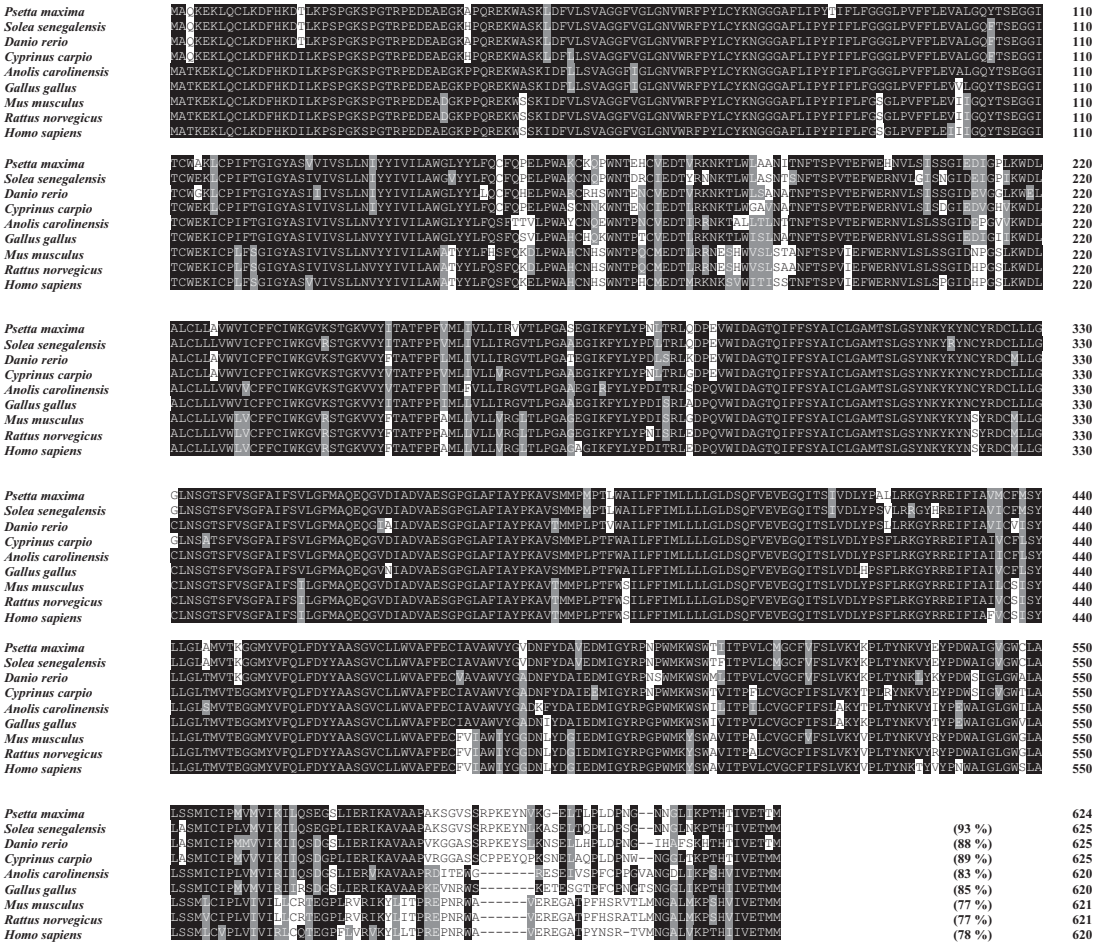

Figure 2 Multiple sequence alignment of TauTs. Highly conserved residues were indicated with black backgrounds and similar residues were shaded. Dashes indicated gaps. The putative phosphorylation site of mammal TauT was boxed. Per cent indentifies of amino acids sequences with *Pm*TauT were shown in parentheses at the end. From top to bottom, the sequences were from *Psetta maxima* (KT369001), *Solea senegalensis* (ADM88612), *Danio rerio* (NP_001119858), *Cyprinus carpio* (BAA89537), *Anolis carolinensis* (XP_003217787), *Gallus gallus* (NP_001025771), *Mus musculus* (NP_033346), *Rattus norvegicus* (AAA42304) and *Homo sapiens* (NP_003034).

Discussion

In this study, we obtained the full coding cDNA sequence of turbot (*P. maxima*) taurine transporter (*Pm*TauT) for the first time. Twelve transmembrane domains were identified in the deduced amino acid sequence of *Pm*TauT, which was in accord with TauTs from other species (Zarate & Bradley 2007; Pinto *et al.* 2012). *Pm*TauT exhibits highly homology to TauTs from other species, which suggests the highly evolutionary conservation of TauT.

Previous studies showed that TauT activity was regulated by phosphorylation through protein kinase C (PKC) (Jones, Miller, Dowling & Chesney 1991; Brandsch, Miyamoto, Ganapathy & Leibach 1993; Loo, Hirsch, Sarkar & Wright 1996; Tappaz 2004). The activation of PKC inhibited the activity of TauT through decreasing the maximal velocity or reducing the number of active transporter in the plasma membrane. Among the putative phosphorylation sites, serine-322 has been proved to be critical for activity regulation through PKC (Han, Budreau & Chesney 1999). Replacement of ser-322 with alanine could increase threefold transport activity of Madin–Darby canine kidney cell taurine transporter, which was through improving the

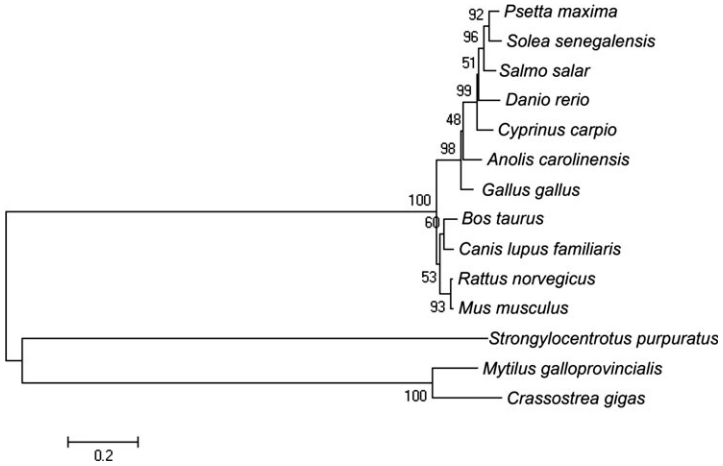

Figure 3 Phylogenetic tree of deduced amino acid sequences of TauT. The tree was constructed using MEGA 4.0 software with the neighbour-joining method. The bootstrap sampling was performed with 1000 replicates. The numbers at the forks indicated the bootstrap. From top to bottom, the sequences were from *Psetta maxima* (KT369001), *Solea senegalensis* (ADM88612), *Salmo salar* (AAM90737), *Danio rerio* (NP_001119858), *Cyprinus carpio* (BAA89537), *Anolis carolinensis* (XP_003217787), *Gallus gallus* (NP_001025771), *Bos taurus* (NP_777035), *Canis lupus familiaris* (NP_001003311), *Rattus norvegicus* (AAA42304), *Mus musculus* (NP_033346), *Strongylocentrotus purpuratus* (XP_011676091), *Mytilus galloprovincialis* (BAD91313) and *Crassostrea gigas* (NP_001292278).

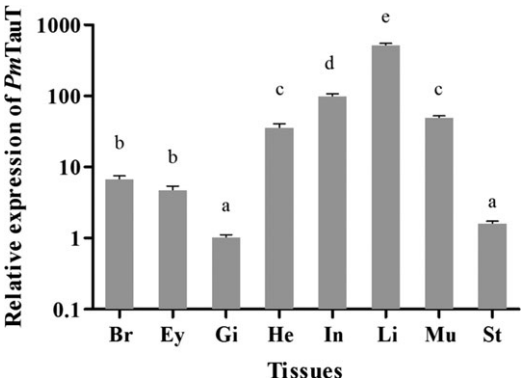

Figure 4 Tissue distribution of TauT in juvenile turbot. Relative *Pm*TauT mRNA levels were measured by quantitative real-time PCR (qRT-PCR) and normalized by GAPDH. The detected tissues were *Br* brain, *Ey* eye, *Gi* gill, *He* heart, *In* intestine, *Li* liver, *Mu* muscle and *St* stomach. The TauT expression levels of Gi was normalized to 1.0 and the rest tissues were expressed as relative expression values to the Gi group. The data were expressed as means ± SEM (n = 6). Different letters above the bars denote significant differences between groups at the $P < 0.05$ level.

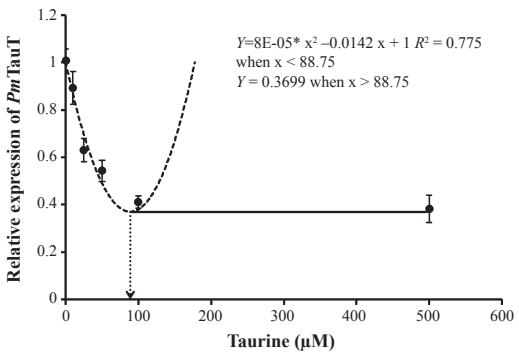

Figure 5 Relative mRNA expression levels of *Pm*TauT in response to taurine concentrations in isolated turbot muscle cells. Turbot muscle cells were isolated and treated with various levels of taurine (0, 10, 25, 50, 100, 500 μM). The *Pm*TauT mRNA levels were measured by quantitative real-time PCR (qRT-PCR) and normalized by GAPDH. The *Pm*TauT expression levels of taurine-free group was normalized to 1.0 and the rest tissues were expressed as relative expression values to it. The data were expressed as means ± SEM (n = 4).

transport capacity V_{max} rather than the affinity K_m (Han *et al.* 2006). Sequence analysis showed that Ser-322 was only conserved in mammal TauTs and this position was occupied by cysteine in fish, reptiles and birds (Fig. 2). Since cysteine could not be phosphorylated by PKC, it indicates that TauTs from non-mammals vertebrates might have a higher transport capacity and the regulation of TauT activity was different between mammals and non-mammalian vertebrates. These

results were also in accord with the phylogenetic tree analysis that TauTs from mammals and other vertebrates fell into two subclusters. However, the regulation of TauT activity in non-mammalian vertebrates needs to be further studied.

TauT was ubiquitously expressed in all examined tissues in juvenile turbot, which was similar with that in other fish species (Takeuchi *et al.* 2000a; Zarate & Bradley 2007; Pinto *et al.* 2012). The expression levels of TauT might be in connected with the physiological roles of taurine in different tissues (Sturman, Rassin & Gaull 1977). In this study, *Pm*TauT was predominantly detected in the tissues of liver and intestine. Liver is the major site for sulphur amino acids metabolism and taurine biosynthesis (Garcia & Stipanuk 1992). Taurine plays a significant role in conjugation with bile acids in liver (Huxtable 1992). However, due to the limited biosynthesis ability of turbot, taurine homoeostasis in liver was also partly dependent on transportation (Wang *et al.* 2014). In the digestive tract of turbot, *Pm*TauT was expressed at a higher level in intestine than stomach. It indicates that intestine was the major site of taurine absorption in juvenile turbot. However, Pinto *et al.* (2012) reported that TauT was actively expressed in stomach of juvenile Senegalese sole and the absorption of dietary taurine might start in the stomach region. These differential results might be due to the different developmental stages or species. In addition, the high expression of TauT in intestine was critical for enterohepatic circulation of taurine in turbot, which was important for maintaining taurine body pool (Hofmann 2008). Moreover, *Pm*TauT was also well detected in muscle, heart, brain and eye. Previous studies have found that taurine was high in these organs and it played many important physiological roles (Huxtable 1992). Interestingly, *Pm*TauT was less expressed in turbot gill, an important organ regulating the internal osmotic pressure (Hoar 1988). As the transporter of taurine, a well-established osmolyte, TauT showed a hypertonicity-induced expression pattern (Takeuchi *et al.* 2000a). The low expression level of *Pm*TauT in turbot gill might indicate that the environment of seawater in this study was suitable for the growth of turbot.

Dietary taurine levels has a significant contribution of the taurine body pool in several fish species, such as Senegalese sole (Pinto *et al.* 2010), red seabream (Takagi, Murata, Goto, Ichiki, Endo, Hatate, Yoshida, Sakai, Yamashita & Ukawa 2006b), Japanese flounder (Kim *et al.* 2008) and turbot (Qi *et al.* 2012; Wang *et al.* 2014). As the conditional essential amino acid for fish, supplementation of taurine to plant protein based aquafeeds has been proved necessary. In this study, the expression level of *Pm*TauT was high when the turbot muscle cells were exposed in taurine-free medium and it was down-regulated by taurine in a dose-dependent manner. Analogous results were also found in early studies (Friedman, Albright, Gusowski, Padilla & Chesney 1983; Bitoun & Tappaz 2000). The regulation of TauT expression by substrate suggests that the capacity of taurine absorption is high when dietary taurine level is low. Our previous study has suggested that hepatic taurine content in different stages of rainbow trout was controlled in a fairly tight range (Wang, He, Mai, Xu & Zhou 2015). Meanwhile, the taurine level in plasma and liver yellowtail did not further increase when the supplemental taurine increased from 30 to 60 g kg^{-1} (Takagi *et al.* 2006a). The sensitive regulation TauT by taurine might be critical for the physiologically effects of taurine (Tappaz 2004). Furthermore, it has been established that the down-regulation of TauT by taurine resulted from a decreased transcription rate of the TauT gene (Han, Budreau & Chesney 1997).

In conclusion, we isolated taurine transporter from turbot and found that *Pm*TauT was down-regulated by taurine in a dose-dependent manner. These results provide an expanded perspective on the homoeostasis of taurine in turbot. Furthermore, this finding may be of vital importance for defining the supplementation of taurine in aquafeeds.

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The authors declare that they have no conflicts of interest with the contents of this article.

References

Al-Feky S.S.A., El-Sayed A.F.M. & Ezzat A.A. (2015) Dietary taurine enhances growth and feed utilization in larval Nile tilapia (*Oreochromis niloticus*) fed soybean meal-based diets. *Aquaculture Nutrition* doi:10.1111/anu.12266. [Epub ahead of print].

Bitoun M. & Tappaz M. (2000) Taurine down-regulates basal and osmolarity-induced gene expression of its transporter, but not the gene expression of its biosynthetic enzymes, in astrocyte primary cultures. *Journal of Neurochemistry* **75**, 919–924.

Bouckenooghe T., Remacle C. & Reusens B. (2006) Is taurine a functional nutrient? *Current Opinion in Clinical Nutrition and Metabolic Care* **9**, 728–733.

Brandsch M., Miyamoto Y., Ganapathy V. & Leibach F.H. (1993) Regulation of taurine transport in human colon carcinoma cell lines (HT-29 and Caco-2) by protein kinase C. *American Journal of Physiology* **264**, G939–G946.

Castillo J., Le Bail P.Y., Paboeuf G., Navarro I., Weil C., Fauconneau B. & Gutierrez J. (2002) IGF-I binding in primary culture of muscle cells of rainbow trout: changes during in vitro development. *American Journal of Physiology Regulatory, Integrative and Comparative Physiology* **283**, R647–R652.

Chatzifotis S., Polemitou I., Divanach P. & Antonopoulou E. (2008) Effect of dietary taurine supplementation on growth performance and bile salt activated lipase activity of common dentex, *Dentex dentex*, fed a fish meal/soy protein concentrate-based diet. *Aquaculture* **275**, 201–208.

Chesney R.W., Gusowski N. & Dabbagh S. (1985) Renal cortex taurine content regulates renal adaptive response to altered dietary intake of sulfur amino acids. *The Journal of clinical investigation* **76**, 2213–2221.

Chow S.C., Ching L.Y., Wong A.M.F. & Wong C.K.C. (2009) Cloning and regulation of expression of the Na^+–Cl^-–taurine transporter in gill cells of freshwater Japanese eels. *Journal of Experimental Biology* **212**, 3205–3210.

Conceição L.E.C., van der Meeren T., Verreth J.A.J., Evjen M.S., Houlihan D.F. & Fyhn H.J. (1997) Amino acid metabolism and protein turnover in larval turbot (*Scophthalmus maximus*) fed natural zooplankton or Artemia. *Marine Biology* **129**, 255–265.

El-Sayed A.-F.M. (2014) Is dietary taurine supplementation beneficial for farmed fish and shrimp? A comprehensive review. *Reviews in Aquaculture* **6**, 241–255.

Espe M. & Holen E. (2013) Taurine attenuates apoptosis in primary liver cells isolated from Atlantic salmon (*Salmo salar*). *The British Journal of Nutrition* **110**, 20–28.

Espe M., Ruohonen K. & El-Mowafi A. (2012) Hydrolysed fish protein concentrate (FPC) reduces viscera mass in Atlantic salmon (*Salmo salar*) fed plant-protein-based diets. *Aquaculture Nutrition* **18**, 599–609.

Felsenstein J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Friedman A.L., Albright P.W., Gusowski N., Padilla M. & Chesney R.W. (1983) Renal adaptation to alteration in dietary amino acid intake. *American Journal of Physiology* **245**, F159–F166.

Garcia R.A. & Stipanuk M.H. (1992) The splanchnic organs, liver and kidney have unique roles in the metabolism of sulfur amino acids and their metabolites in rats. *The Journal of Nutrition* **122**, 1693–1701.

Goto T., Matsumoto T., Murakami S., Takagi S. & Hasumi F. (2003) Conversion of cysteate into taurine in liver of fish. *Fisheries Science* **69**, 216–218.

Han X., Budreau A.M. & Chesney R.W. (1997) Adaptive regulation of MDCK cell taurine transporter (pNCT) mRNA: transcription of pNCT gene is regulated by external taurine concentration. *Biochimica et Biophysica Acta* **1351**, 296–304.

Han X., Budreau A.M. & Chesney R.W. (1999) Ser-322 is a critical site for PKC regulation of the MDCK cell taurine transporter (pNCT). *Journal of the American Society of Nephrology* **10**, 1874–1879.

Han X., Patters A.B., Jones D.P., Zelikovic I. & Chesney R.W. (2006) The taurine transporter: mechanisms of regulation. *Acta Physiologica (Oxford, England)* **187**, 61–73.

Hedges S.B. (1992) The number of replications needed for accurate estimation of the bootstrap P value in phylogenetic studies. *Molecular Biology and Evolution* **9**, 366–369.

Hoar W.S. (1988) The physiology of smolting salmonids. In: *Fish Physiology* (ed. by W.S. Hoar & D.J. Randall), pp. 275–343. Academic Press, New York, USA.

Hofmann A.F. (2008) The enterohepatic circulation of bile acids in mammals: form and functions. *Frontiers in Bioscience* **14**, 2584–2598.

Huxtable R.J. (1992) Physiological actions of taurine. *Physiological Reviews* **72**, 101–163.

Jayanthi L.D., Ramamoorthy S., Mahesh V.B., Leibach F.H. & Ganapathy V. (1995) Substrate-specific regulation of the taurine transporter in human placental choriocarcinoma cells (JAR). *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1235**, 351–360.

Jones D.P., Miller L.A., Dowling C. & Chesney R.W. (1991) Regulation of taurine transporter activity in LLC-PK1 cells: role of protein synthesis and protein kinase C activation. *Journal of the American Society of Nephrology* **2**, 1021–1029.

Kato K., Yamamoto M., Peerapon K., Fukada H., Biswas A., Yamamoto S., Takii K. & Miyashita S. (2014) Effects of dietary taurine levels on epidermal thickness and scale loss in red sea bream, *Pagrus major*. *Aquaculture Research* **45**, 1818–1824.

Kaushik S. & Luquet P. (1977) Study of free amino acids in rainbow trout in relation. *Annales D Hydrobiologie* **8**, 135–144.

Kim S.-K., Matsunari H., Takeuchi T., Yokoyama M., Murata Y. & Ishihara K. (2007) Effect of different dietary taurine levels on the conjugated bile acid composition and growth performance of juvenile and fingerling Japanese flounder *Paralichthys olivaceus*. *Aquaculture* **273**, 595–601.

Kim S.K., Matsunari H., Takeuchi T., Yokoyama M., Furuita H., Murata Y. & Goto T. (2008) Comparison of taurine biosynthesis ability between juveniles of Japanese flounder and common carp. *Amino Acids* **35**, 161–168.

Kozlowski D.J., Chen Z., Zhuang L., Fei Y.-J., Navarre S. & Ganapathy V. (2008) Molecular characterization and expression pattern of taurine transporter in zebrafish during embryogenesis. *Life Sciences* **82**, 1004–1011.

Kuzmina V.V., Gavrovskaya L.K. & Ryzhova O.V. (2010) Taurine. Effect on exotrophia and metabolism in mammals and fish. *Journal of Evolutionary Biochemistry and Physiology* **46**, 19–27.

Li P., Mai K., Trushenski J. & Wu G. (2009) New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids* **37**, 43–53.

Lim S.-J., Oh D.-H., Khosravi S., Cha J.-H., Park S.-H., Kim K.-W. & Lee K.-J. (2013) Taurine is an essential nutrient for juvenile parrot fish *Oplegnathus fasciatus*. *Aquaculture* **414–415**, 274–279.

Livak K.J. & Schmittgen T.D. (2001) Analysis of relative gene expression data using real-time quantitative pcr and the $2^{-\Delta\Delta Ct}$ method. *Methods* **25**, 402–408.

Loo D.D.F., Hirsch J.R., Sarkar H.K. & Wright E.M. (1996) Regulation of the mouse retinal taurine transporter (TAUT) by protein kinases in Xenopus oocytes. *FEBS Letters* **392**, 250–254.

Lunger A.N., McLean E., Gaylord T.G., Kuhn D. & Craig S.R. (2007) Taurine supplementation to alternative dietary proteins used in fish meal replacement enhances growth of juvenile cobia (*Rachycentron canadum*). *Aquaculture* **271**, 401–410.

Maita M., Maekawa J., Satoh K.-I., Futami K. & Satoh S. (2006) Disease resistance and hypocholesterolemia in yellowtail *Seriola quinqueradiata* fed a non-fishmeal diet. *Fisheries Science* **72**, 513–519.

O'Flaherty L., Stapleton P.P., Redmond H.P. & Bouchier-Hayes D.J. (1997) Intestinal taurine transport: a review. *European Journal of Clinical Investigation* **27**, 873–880.

Pinto W., Figueira L., Ribeiro L., Yúfera M., Dinis M.T. & Aragão C. (2010) Dietary taurine supplementation enhances metamorphosis and growth potential of *Solea senegalensis* larvae. *Aquaculture* **309**, 159–164.

Pinto W., Rønnestad I., Jordal A.-E., Gomes A., Dinis M. & Aragão C. (2012) Cloning, tissue and ontogenetic expression of the taurine transporter in the flatfish Senegalese sole (*Solea senegalensis*). *Amino Acids* **42**, 1317–1327.

Qi G., Ai Q., Mai K., Xu W., Liufu Z., Yun B. & Zhou H. (2012) Effects of dietary taurine supplementation to a casein-based diet on growth performance and taurine distribution in two sizes of juvenile turbot (*Scophthalmus maximus L.*). *Aquaculture* **358–359**, 122–128.

Rozen R. & Scriver C.R. (1982) Renal transport of taurine adapts to perturbed taurine homeostasis. *Proceedings of the National Academy of Sciences of the United States of America* **79**, 2101–2105.

Saitou N. & Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406–425.

Salze G.P. & Davis D.A. (2015) Taurine: a critical nutrient for future fish feeds. *Aquaculture* **437**, 215–229.

Salze G., Craig S.R., Smith B.H., Smith E.P. & McLean E. (2011) Morphological development of larval cobia *Rachycentron canadum* and the influence of dietary taurine supplementation. *Journal of Fish Biology* **78**, 1470–1491.

Sambrook J. & Russell David W. (1989) *Molecular Cloning: A Laboratory Manual*, Vol. 3. Cold Spring Harbor Laboratory Press, New York, USA.

Schuller-Levis G.B. & Park E. (2003) Taurine: new implications for an old amino acid. *FEMS Microbiology Letters* **226**, 195–202.

Sturman J.A., Rassin D.K. & Gaull G.E. (1977) Taurine in development. *Life Sciences* **21**, 1–21.

Tacon A.G.J. & Metian M. (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture* **285**, 146–158.

Takagi S., Murata H., Goto T., Hayashi M., Hatate H., Endo M., Yamashita H. & Ukawa M. (2006a) Hemolytic suppression roles of taurine in yellowtail *Seriola quinqueradiata* fed non-fishmeal diet based on soybean protein. *Fisheries Science* **72**, 546–555.

Takagi S., Murata H., Goto T., Ichiki T., Endo M., Hatate H., Yoshida T., Sakai T., Yamashita H. & Ukawa M. (2006b) Efficacy of taurine supplementation for preventing green liver syndrome and improving growth performance in yearling red sea bream *Pagrus major* fed low-fishmeal diet. *Fisheries Science* **72**, 1191–1199.

Takagi S., Murata H., Goto T., Endo M., Yamashita H. & Ukawa M. (2008) Taurine is an essential nutrient for yellowtail *Seriola quinqueradiata* fed non-fish meal diets based on soy protein concentrate. *Aquaculture* **280**, 198–205.

Takeuchi K., Toyohara H., Kinoshita M. & Sakaguchi M. (2000a) Ubiquitous increase in taurine transporter mRNA in tissues of tilapia (*Oreochromis mossambicus*) during high-salinity adaptation. *Fish Physiology and Biochemistry* **23**, 173–182.

Takeuchi K., Toyohara H. & Sakaguchi M. (2000b) A hyperosmotic stress-induced mRNA of carp cell

encodes Na^+- and Cl^--dependent high affinity taurine transporter1. *Biochimica et Biophysica Acta (BBA) – Biomembranes* **1464**, 219–230.

Tappaz M.L. (2004) Taurine biosynthetic enzymes and taurine transporter: molecular identification and regulations. *Neurochemical Research* **29**, 83–96.

Wang Q., He G., Wang X., Mai K., Xu W. & Zhou H. (2014) Dietary sulfur amino acid modulations of taurine biosynthesis in juvenile turbot (*Psetta maxima*). *Aquaculture* **422–423**, 141–145.

Wang X., He G., Mai K., Xu W. & Zhou H. (2015) Ontogenetic taurine biosynthesis ability in rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **185**, 10–15.

Yokoyama M., Takeuchi T., Park G.S. & Nakazoe J. (2001) Hepatic cysteinesulphinate decarboxylase activity in fish. *Aquaculture Research* **32**, 216–220.

Yun B., Ai Q., Mai K., Xu W., Qi G. & Luo Y. (2012) Synergistic effects of dietary cholesterol and taurine on growth performance and cholesterol metabolism in juvenile turbot (*Scophthalmus maximus L.*) fed high plant protein diets. *Aquaculture* **324–325**, 85–91.

Zarate J.M. & Bradley T.M. (2007) Molecular cloning and characterization of the taurine transporter of Atlantic salmon. *Aquaculture* **273**, 209–217.