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Characterization and expression profiles of aquaporins (AQPs) 1a and 3a in mud loach *Misgurnus mizolepis* after experimental challenges

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Abstract

Two distinct cDNAs encoding aquaporins (mmAQPs 1a and 3a) were isolated and characterized from mud loach *Misgurnus mizolepis*. The identified mud loach AQP cDNAs encode for polypeptides of 260 and 302 amino acids. Topology predictions confirmed six putative membrane-spanning domains connected by five loops and the N- and C-terminal domains being cytoplasmic. The mud loach AQPs 1a and 3a showed broad distribution in multiple tissues including immune-responsive tissues as well as osmoregulatory tissues. Hence, the diversity of AQP distribution and expression possibly indicated its differential functions in the regulation of fluid movement in response to environmental stimuli. The transcription of mmAQP genes was differentially modulated by immune challenges. In particular, the mmAQP3a expression level in the liver was more responsive to immune challenges than that of mmAQP1a. Taken together, fish stimulation or infection resulted in significant modulation of mud loach AQP genes, suggesting potential functional roles of these proteins in piscine pathophysiological process.

Keywords: *Misgurnus mizolepis*, Aquaporins, Lipopolysaccharide, Polyinosinic: polycytidylic acid, Bacterial infection

Background

Teleostean species possess osmoregulatory system and therefore can overcome salt and water balances along with environmental fluctuations. The epithelia such as gill or intestine are the main site for sensing changes in the salinity, contribute to the alleviation of osmotic stress, and occasionally harbor mechanism to avoid infection (Fiol and Kültz 2007). According to change in the surrounding environment, the macromolecules, such as proteins, trigger complex responses, e.g., cell volume change, changes in cytoskeletal organization or whole tissue remodeling (Fiol and Kültz 2007; Henry et al. 2003).

Aquaporins (AQPs) are important mediators of the movement of water and other small solutes and cell volume regulation. At least 13 aquaporin isoforms have been identified in different organisms ranging from

bacteria to humans (Kozono et al. 2003; King et al. 2004) and are categorized into three subfamilies on the basis of their substrate permeability: water-selective aquaporins, aquaglyceroporin (which is permeable to glycerol and certain small, uncharged solutes), and unorthodox subgroup (Ishibashi et al. 2011). The AQPs are ubiquitously expressed in a number of somatic tissues such as the gills, kidney, eye, skeletal muscle, and lung. In addition to their well-established osmoregulatory function, AQPs appear to play important roles in physiological processes including neural signal transduction, skin hydration, brain swelling, and cell migration (Zhu et al. 2011; Verkman 2012). Furthermore, considering its presence in immune-mediated cell, AQPs might also have potential functional role in linkage between unfavorable water flux through its activities and the epithelial barrier immune system (Zhu et al. 2011).

The existence of AQP expression in different tissues clearly indicates its potential functional role in a wide range of biological processes (Gomes et al. 2009;

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Watanabe et al. 2009; Boj et al. 2015). In freshwater prawn *Macrobrachium rosenbergii*, change in salinity has showed a direct or indirect effect on the respiratory metabolism, mortality, growth, and even immune response later (Cheng and Chen 2000; Moshtaghi et al. 2016). To date, extensive studies have been conducted on a number of fish species to investigate tissue-specific functional roles of AQPs under different salinity environments (Cutler and Cramb 2000; Watanabe et al. 2005; Giffard-Mena et al. 2007; Kim et al. 2010; Choi et al. 2013). In addition, many AQP isoforms have been detected in tissues that are not directly involved in osmoregulation, as evidenced by several studies (Watanabe et al. 2009; Kim et al. 2014; Madsen et al. 2014; Boj et al. 2015). However, functional roles of piscine aquaporin genes with regard to immune or bacterial challenges have still remained unexplored. The limited information may hinder a comprehensive understanding on the coordinated role of AQP isoforms in the maintenance of physiological homeostasis.

Mud loach *Misgurnus mizolepis* (Teleostei; Cypriniformes) is a promising candidate for freshwater aquaculture whose market demand is gradually increasing in Korea. In addition to its commercial importance, mud loach has the potential to be used as an experimental model animal for investigating various types of theoretical and practical issues. Some interesting biological features of this species include small adult size, high fecundity, year around spawning capability under controlled conditions, and relatively well-established techniques for its genetic manipulation (Nam et al. 2011; Cho et al. 2012). Considering these facts, mud loach could be an ideal model for investigating the functional roles of the AQP-mediated cellular process in the pathophysiological situation.

Edwardsiella tarda was chosen for this study because it has been demonstrated as a potential pathogen capable of causing disease and stimulating the immune responses in mud loach (Lee et al. 2011; Nam et al. 2011). In the species most commonly associated with *E. tarda* infection, the disease is a generalized septicemia with clinical sign including extensive skin lesions, bloody ascites in abdominal cavity, and damage to internal organs (Plumb 1999).

In line with our long-term goal for comprehensive understanding on the osmophysiology and innate immunity of mud loach, the objective of this study was to examine the expression patterns of AQP genes with regard to immunostimulant or bacterial infection. In the current study, we focused on AQPs 1a and 3a from mud loach, genetically characterized two aquaporin isoforms, and examined their expression patterns in response to immune stimulatory challenges.

Methods

Isolation of mud loach aquaporin 1a and 3a cDNAs

Mud loach *M. mizolepis* expressed sequence tag database (conducted from total RNA) was surveyed to isolate the cDNA sequence for aquaporin. Several partial mud loach AQP clones showing the high homology to previously known vertebrate AQPs were identified using a homology search in NCBI GenBank. Based on the contig assemblies using Sequencher software (Gene Codes Co., Ann Arbor, MI, USA), two distinct isoforms (designated mmAQP1a and mmAQP3a) were identified from various tissues. The full-length cDNA sequence of each aquaporin gene was confirmed from RT-PCR and/or vectorette PCR with an SK vector primer (Stratagene, La Jolla, Ca, USA) and specific primer pair sets (mmAQP1a FW/RV [for *mmAQP1a*] or mmAQP3a FW/RV [for *mmAQP3a*]) (Table 1). The representative

Table 1 List of oligonucleotide primers used in this study

Primer name	Sequence (5'-3')	Thermal cycling conditions	Application
mmAQP1a FW	CAGAAATCTCCACATTACCAGC	35 cycles at 94 °C for 20 s, 58 °C for 20 s, 72 °C for 1.5 min, followed by a final elongation at 72 °C for 3 min	Isolation of mud loach aquaporin cDNAs
mmAQP1a RV	TCCTGAGGTACATACTGATTC		
mmAQP3a FW	ACACACGTTCAAGGGAAGC		
mmAQP3a RV	CTGGCTGGATTCACAGCATA		
mm18S rRNA RV	GGTTTCCCGTGTGAGTCAA	Reverse transcription at 37 °C for 60 min	Preparation of normalization control
q-mmAQP1a FW	ATGAGAGTGCTGGTCTCTGG	45 cycles at 94 °C for 20 s, 60 °C for 20 s and 72 °C for 20 s	Real-time PCR assay of mud loach aquaporin mRNAs
q-mmAQP1a RV	AAAGACAGCTTCACAATTGC		
q-mmAQP3a FW	TATGGAGGAGAATGTGAAGC		Real-time PCR assay of 18S rRNA control
q-mmAQP3a RV	AATCTGGAGATGTGCAGCGT		
q-mm18S rRNA FW	AAGCTCGTAGTTGGATCTCG		
q-mm18S rRNA RV	CCTAGCTGCGGTATTCAAGC		

Each PCR amplification reaction was performed with an initial denaturation step at 94 °C for 2 min

cDNA sequences for each AQP isoform were determined with multiple PCR clones ($n \geq 6$) and/or amplified PCR products.

Bioinformatic sequence analysis

Protein-coding sequences for each AQP isoform were obtained using the open reading frame (ORF) finder (<https://www.ncbi.nlm.nih.gov/orffinder/>) (Wheeler et al. 2003). The molecular mass and theoretical isoelectric point (pI) value were computed using ExPASy ProtParam tool for each isoform (<http://web.expasy.org/protparam/>) (Gasteiger et al. 2005). We find the corresponding teleostean and human AQPs from BLAST and/or the Ensembl genome browser (<http://www.ensembl.org/index.html>) to examine their homology. ORFs of mud loach aquaporin were aligned with teleostean and human orthologues using the multiple sequence alignment programs CLUSTAL W or CLUSTAL X 1.81 (Thompson et al. 1994; Chenna et al. 2003). The information about GenBank accession numbers or Ensembl codes for aquaporin sequences are provided in Table 2. Topology prediction for deduced amino acid sequences of AQPs was performed with the software TMHMM (<http://www.cbs.dtu.dk/services/TMHMM/>) (Krogh et al. 2001).

Preparation of tissue samples for basal expression assay

For the tissue distribution assay of mmAQP transcripts, ten somatic tissues (brain, eye, fin, gill, heart, intestine, kidney, liver, skeletal muscle, and spleen) and two gonad tissues (ovary and testis) were derived from 12 healthy individuals (average body weight = 9.3 ± 2.5 g). Upon surgically removed, biological samples were immediately frozen on dry ice and stored at -80 °C until use.

Immune challenges

To examine whether the expression of mmAQP genes is induced by inflammatory stimuli and immune challenge, lipopolysaccharide (LPS; *Escherichia coli* 0111:B4) or polyinosinic: polycytidylic acid [poly(I:C)] injection and bacterial challenge (*E. tarda*; Gram negative) (Kwon et al. 2005) were performed in vivo. First, LPS (Sigma-Aldrich, St Louis, MO, USA; 5 µg/g body weight [BW]) or poly(I:C) (Sigma-Aldrich, 25 µg/g BW) was injected intraperitoneally into fish individuals (10.5 ± 2.1 g; $n = 8$ for each group). Saline control ($n = 8$) were done with this study. Injection volume was 100 µL. After injection, each group was allocated into each 60-L tanks at 25 °C and no feed was supplied during experimental period. For LPS and poly(I:C) challenges, immune-relevant

Table 2 Amino acid sequence identities of mud loach AQPs 1a and 3a with other orthologues

	Species	Gene name	Accession no.	Identity (%)	
mmAQP1a	<i>Oryzias dancena</i>	AQP1	AB759557	80	
	<i>Osmerus mordax</i>	AQP1a	ACO09149	80	
	<i>Danio rerio</i>	AQP1a	NP_996942	87	
	<i>Acanthopagrus schlegelii</i>	AQP1a	ABO38816	80	
	<i>Dicentrarchus labrax</i>	AQP1a	ABI95464	79	
	<i>Fundulus heteroclitus</i>	AQP1a	ACI49538	80	
	<i>Danio rerio</i>	AQP1b	NP_001129154	63	
	<i>Solea senegalensis</i>	AQP1b	AAV34612	57	
	<i>Salmo salar</i>	AQP1b	ACI33306	72	
	<i>Sparus aurata</i>	AQP1b	EF011740	61	
	<i>Anguilla anguilla</i>	AQP1b	CAD92028	63	
	<i>Homo sapiens</i>	AQP1	BAG70089	60	
	mmAQP3a	<i>Oryzias dancena</i>	AQP3	AB759557	70.4
		<i>Danio rerio</i>	AQP3a	EU341833	84.8
<i>Oreochromis mossambicus</i>		AQP3a	BAD20708	72.2	
<i>Dicentrarchus labrax</i>		AQP3a	ABG36519	69.9	
<i>Fundulus heteroclitus</i>		AQP3a	ACI49539	68.8	
<i>Danio rerio</i>		AQP3b	EU341832	67.9	
<i>Anguilla anguilla</i>		AQP3b	CAC85286	73.6	
<i>Anguilla japonica</i>		AQP3	BAH89253	72	
<i>Astyanax mexicanus</i>		AQP3	XP_007238017	69	
<i>Homo sapiens</i>		AQP3	AB001325	64.7	

tissues (kidney, intestine, liver, and spleen) were surgically removed from three individuals in each group at 24 h post challenge.

On other hand, the bacterial challenge was carried out by injecting 1×10^6 cell of *E. tarda* suspended in 100 μ L of phosphate-buffered saline (PBS, pH 7.4) intraperitoneally into each individual. Twenty-four individuals (same-size as above) were given *E. tarda* injection, and other 24 individuals were given PBS alone as a control group. Each group (*E. tarda*- or PBS-injected) was transferred to each 100-L tank at 25 °C, and tissue samples were obtained individually from three randomly chosen fish belonging to each tank at 24, 48, and 72 h post injection, respectively. Tissue samples were harvested, frozen, and stored as described above.

RT-PCR analysis of mmAQP transcripts

Total RNA was extracted from dissected tissues using the RNeasy® Plus Micro Kit (Qiagen, Hilden, Germany) including DNase I treatment step. An aliquot of the total RNA (2 μ g) was reverse transcribed into cDNA in a reaction volume of 20 μ l using the Omniscript® Reverse Transcription Kit (Qiagen). The reverse transcription reaction include an oligo-d(T)₂₀ primer (1 μ M final concentration) and a mud loach 18S rRNA (0.1 μ M) reverse primer, as described by Cho et al. (2012). The use of 18S rRNA as the internal standard can be a valuable alternative for quantifying genes of interest, but it may reduce the variation of expression.

The reaction conditions were performed according to the manufacturer's protocol. RT-PCR reactions were performed in 25- μ L reaction volumes including 2- μ l cDNA, 2- μ l primers, 6.5- μ l distilled water, and 12.5- μ l 2 \times iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA). Reaction performed on the iCycler iQ Real-time Detection System (Bio-Rad). The plasmid DNAs containing the amplified parts of target mRNAs were prepared as standard samples. The 231-bp (for AQP1a) and 208-bp (for AQP3a) aquaporin gene fragments were amplified with the specific primer pairs, q-mmAQP1a-FW/RV and q-mmAQP3a-FW/RV, respectively (Table 1). Basal expression level of AQP transcripts in tissue types was represented based on Δ Ct (Ct of the AQP gene subtracted from the Ct of the 18 s RNA gene). On the other hand, the relative expressions of AQP transcripts in the stimulated groups were expressed as the fold change to non-treated control by using the formula $2^{-\Delta\Delta Ct}$ (Kubista et al. 2006; Schmittgen and Livak 2008). Each reaction was performed at least in triplicates.

Statistical analysis

Numerical data were expressed as means \pm S.E.M. Statistical differences were determined by one-way analysis of variance (ANOVA), followed by Duncan's multiple range

tests. All statistical analysis were performed using software SPSS version 10.0 (SAS Inc., Cary, NC, USA), and difference was considered to be significant when $P < 0.05$.

Results

Characteristics of mud loach AQP cDNA 1a and 3a isoforms

We isolated two distinct aquaporin genes from the mud loach. They are 1230 and 1609 bp in the length and contain ORFs of 780 and 906 bp (excluding the termination codon) encoding a polypeptides of 287 and 306 amino acid, respectively. The calculated molecular masses of each isoform are 27.3 and 32.8 kDa, with theoretical pI values of 6.05 and 6.15, respectively. The nucleotide sequences of the two AQP cDNA sequences have been assigned on GenBank under the accession numbers AB971265 (mmAQP1a) and AB971266 (mmAQP3a). The mud loach AQP isoforms showed very lower level of sequence homology (18%) with each other. From multiple sequence alignments with other orthologs, the amino acid sequence of mud loach AQPs shared considerable identities with those from teleostean AQPs (Table 2). The basic features of typical AQP channel structure were observed in mud loach AQP1a and AQP3a isoforms. According to topology prediction for these proteins, those proteins possess six potential transmembrane helices connected by five loops. Both the amino and carboxyl termini are extended into the cytoplasmic side (Fig. 1). Importantly, the two identical asparagine-proline-alanine (NPA) motifs are located in the second and fifth loops, which are the pore-forming signature motifs for AQPs playing a crucial role in the water uptake (Ishibashi et al. 2011). In mmAQP1a, a cysteine residue in position 180, proximal to the C-terminal NPA motif, is possibly pivotal for the inhibition of water permeability by mercurial reagent (Preston et al. 1993).

Basal expression assay of mud loach AQPs 1a and 3a

Under the present RT-PCR conditions, AQP1a and AQP3a transcripts were ubiquitously distributed in all tissue types examined, although the basal levels were significantly different among tissues. In particular, the mRNA levels of AQP1a were higher in non-osmoregulatory tissues, brain, eye, heart, and spleen than osmoregulatory tissues, for example, the intestine, kidney, and gill. On the other hand, AQP3a transcripts in non-stimulated fish were detectable markedly in fin, followed by gill, eye and intestine (Fig. 2).

AQP gene expression profiles after immune challenge

The mud loach AQP1a mRNA was rapidly induced by LPS injection in all tissues, and the fold change relative to the expression level in the saline-injected control was

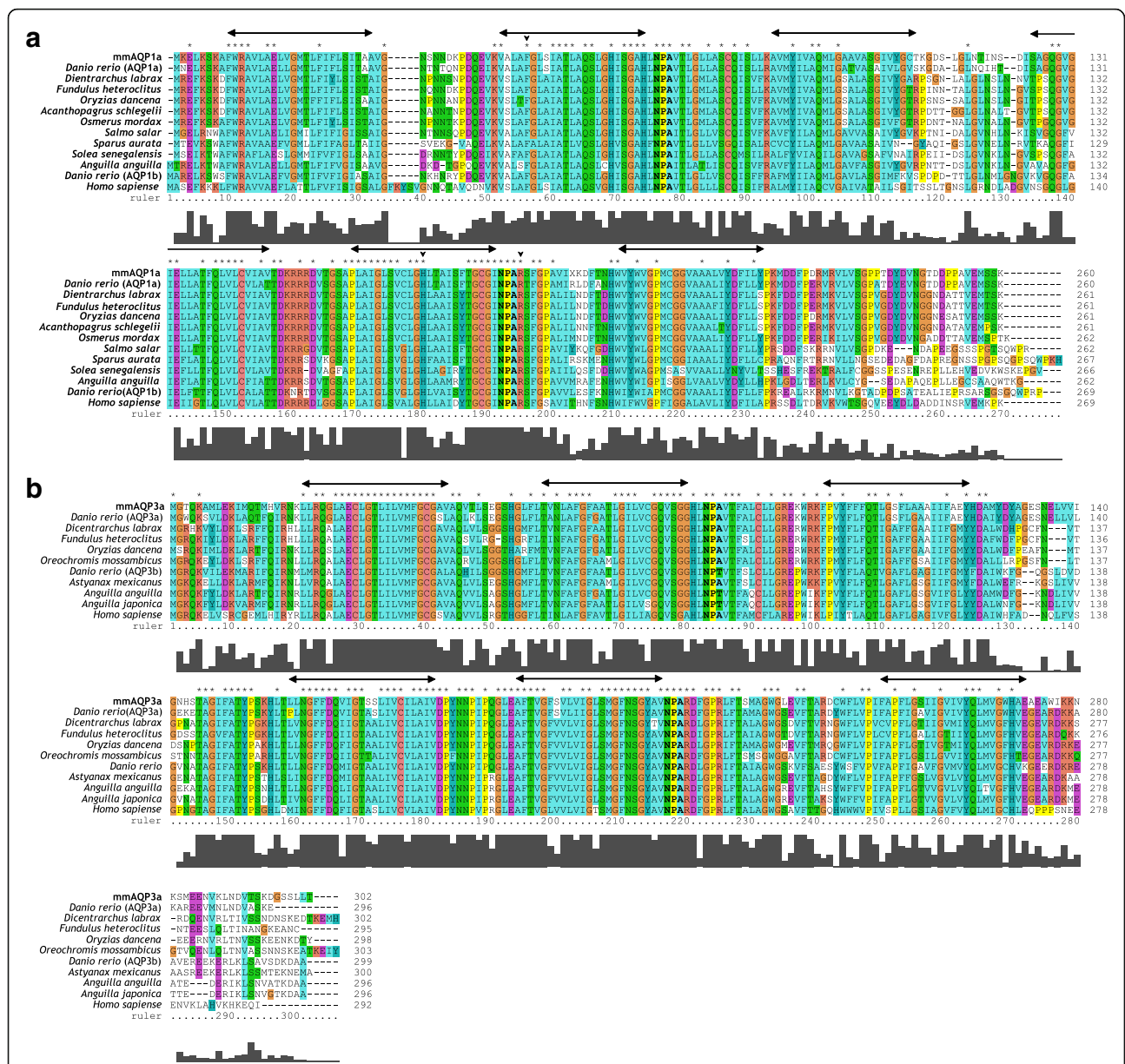


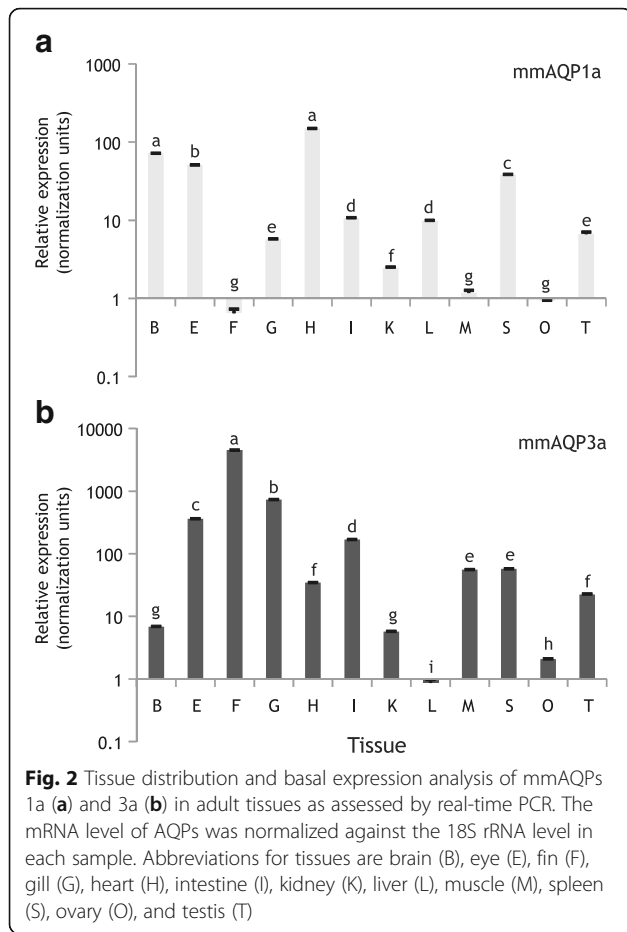
Fig. 1 Multiple amino acid sequence alignments of mud loach *Misgurnus mizolepis* AQP isoforms 1a (a) and 3a (b) along with those from other selected vertebrate species. Asterisks and hyphens indicate identical residues and gaps introduced for optimum alignments, respectively. The arrowhead above mmAQP1a showed the conserved residues Phe⁵⁶, His¹⁸⁰, and Arg¹⁹⁵ (mud loach AQP1a numbering). Two NPA motifs are shown in bold letters. The putative transmembrane locations of membrane-spanning domains are indicated above the alignment. The gray histogram below the ruler depicts sequence conservation between amino acid residues in given AQP isoforms

higher in the liver (1.3-fold) than in the intestine (0.2-fold), kidney (0.5-fold), and spleen (0.4-fold) (Fig. 3a). Also, the transcriptional response of AQP1a to poly(I:C) at 24 h post-injection showed levels highest in the kidney (1.6-fold) compared with other tissues (Fig. 3a). In contrast, the LPS injection significantly stimulated the transcription of AQP3a, which were greatest in the intestine (15.9-fold), followed by the liver (6.14-fold) and spleen (1.05-fold) (Fig. 3b). Moreover, the liver showed a

predominant increase in mmAQP3a transcripts of 23.38-fold induction by poly(I:C) stimulation.

AQP gene profiles after bacterial challenge

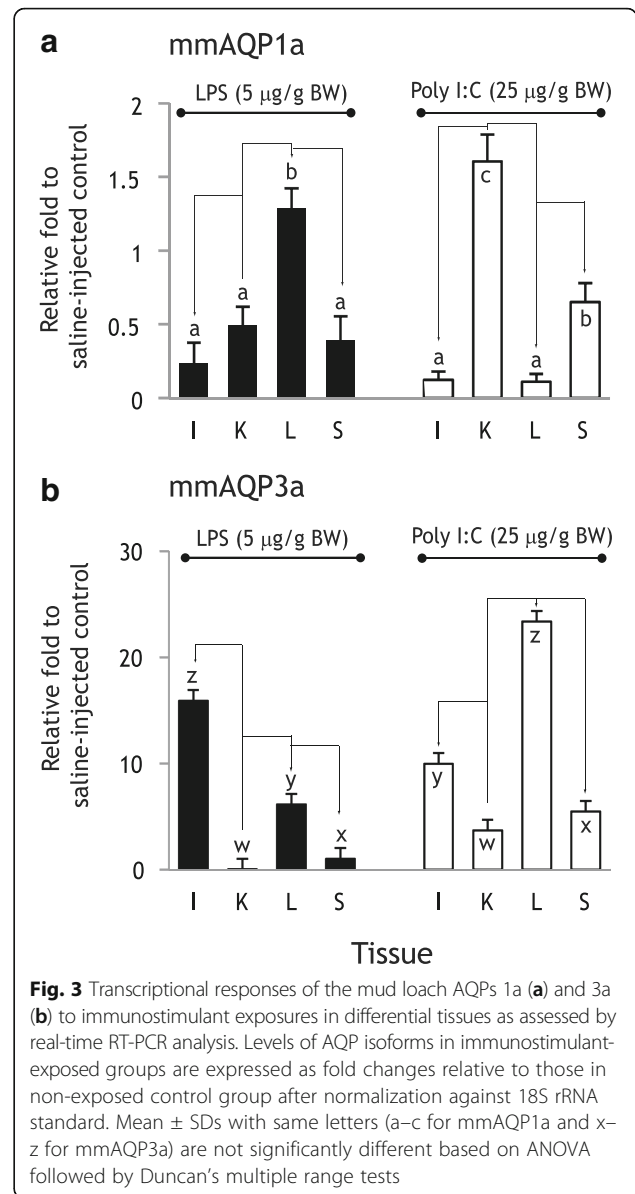
An in vivo bacterial injection was conducted with the known pathogen *E. tarda* (Gram negative; FSW910410), which causes edwardsiellosis in mud loach (Fig. 4). The expression patterns of mud loach AQP1a and AQP3a transcripts differed in the intestine, kidney, liver, and



spleen. The transcriptions of mud loach AQP1a and AQP3a in the intestine were significantly decreased by *E. tarda* challenges, and the reduction of intestinal AQP1a and AQP3a expressions was clearly time-dependent. Compared to that of intestinal response, in the kidney, the expression of the two isoform genes was modulated in opposite directions at each time point examined. In the liver, AQP1a isoform was less modulated by bacterial injection; no significant changes were evident during experimental period; instead, the mmAQP3a transcripts were responsive to *E. tarda* challenges, and the maximum induction of AQP3a expression was observed at 72 h after bacterial injection. The spleen showed the differential induction of the two AQP transcripts during *E. tarda* challenge in this study. The splenic mRNA levels of mmAQP1a were highly elevated (up to seven fold relative to the saline-injected controls) at 48 h post injection (hpi) and rapidly decreased at 72 hpi. In contrast, the mmAQP3a transcripts were slightly but significantly induced in time-dependent manner.

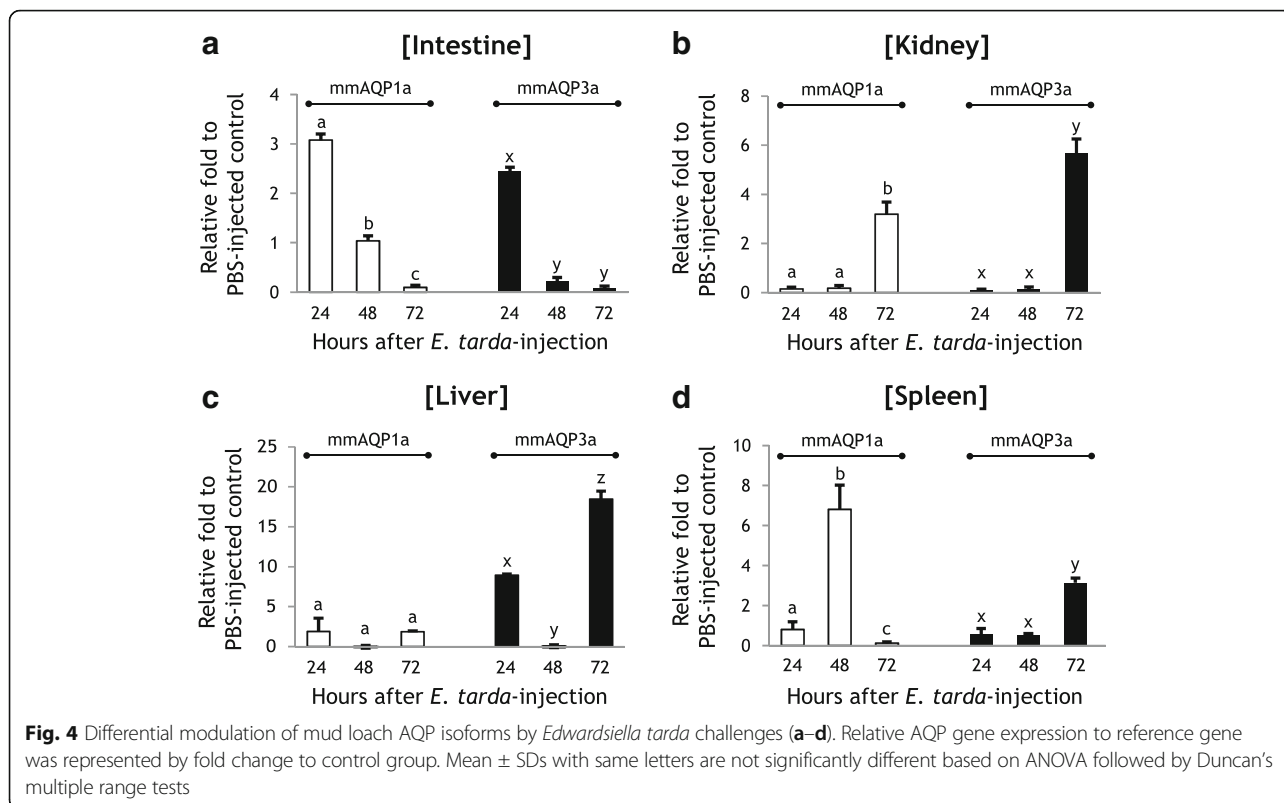
Discussion

We cloned two cDNAs encoding AQPs 1a and 3a from mud loach. The identified AQPs possess six



transmembrane domains which are found in known major intrinsic protein (MIP) structures as well as among aquaporin (Borgnia et al. 1999). The traditional NPA sequences have been harbored to form a characteristic pore between the membrane bilayer (Nielsen et al. 1999). These findings suggest that AQPs from mud loach function as water channels that facilitate passage of water and other small solutes through membrane, although we did not investigate the functional role in detail.

In the present study, transcripts encoding AQP1a were detectable in a wide array of tissues of mud loach, which has also been observed in marine medaka *Oryzias danconena*, Japanese medaka *O. latipes*, zebrafish, Japanese eel *Anguilla japonica* and black porgy, *Acanthopagrus*



schlegeli (An et al. 2008; Tingaud-Sequeira et al. 2010; Kim et al. 2010, 2014; Madsen et al. 2014). Relatively higher mRNA expression levels were observed in the heart, brain, eye, spleen, and intestine but also present in lower levels in all other tissues examined, which in part may manifest a general expression in erythrocytes and endothelial barriers (Mobasheri and Marples 2004). The higher expression of cardiac mmAQP1a may be a main determinant of its role in myocardial fluid balance, as suggested by Japanese eel and silver sea bream *Sparus sarba* (Aoki et al. 2003; Deane et al. 2011). The splenic AQP has been considered to be involved in the trafficking of hematopoietic cells (Tyagi and Tangevelu 2010). The branchial AQP1a showed lower expression level, compared to those of osmoregulatory tissues. This result is somewhat contradictory to previous report that the gills of freshwater fish are the main pathway for water transport and can account for approximately 90% of the total body water influx (Cutler and Cramb 2000; Deane et al. 2011). Although specific mechanism should be investigated in the further study, the branchial AQP1a might act in concert with other homologs (e.g., AQP3a in this study) to prevent cell swelling. The testicular expression of mmAQP1a was noticeable, while lower expressed in ovary. In gilthead sea bream *Sparus aurata*, the distinct aquaporin paralogs (e.g., AQPs 0a, 1a, 1ab, 7, 8b, 9b, and 10b) were involved in control of the fluid

balance during spermatogenesis (Boj et al. 2015). Hence, these facts suggest that testicular function in the mud loach is potentially associated with fine-tuned water control by aquaporin channel.

The observation of AQP3a in mud loach was comparable to that described for several teleost (Deane and Woo 2006; Tingaud-Sequeira et al. 2010; Kim et al. 2014). With reference to expression levels, mmAQP3a mRNAs were unequivocally predominant in the fin that is not primarily involved in osmoregulatory systems, which could be due to thin epidermis. The pattern observed in this study is similar to that reported in tilapia and medaka (Watanabe et al. 2005; Kim et al. 2014; Madsen et al. 2014). Another noticeable feature in mmAQP3a mRNA levels was observed in gill and eye, which are externally exposed organs in fish. Relatively higher AQP3a abundance in the gill suggested its involvement in possible osmoreception by mitochondrion-rich chloride cells (Watanabe et al. 2005). In addition, relatively high level was also found in the spleen, in accordance with several teleost species (Watanabe et al. 2005; Kim et al. 2014). However, mmAQP3a mRNA expression was found at very low level in liver, known as major detoxification organ. This expression pattern is similar to that observed previously in other teleostean fish (Watanabe et al. 2005; Tingaud-Sequeira et al. 2010; Madsen et al. 2014). Thus, the mud loach AQPs 1a and

3a, in common with other teleostean fish AQPs, exhibited broad distribution in multiple diverse tissues including immune-responsive and mucosal tissues exposed to external environment. Hence, the variety of AQP distribution and expression presumably suggested its differential functions in the regulation of water movement according to environmental stimuli.

Overall, the RT-PCR quantitative analysis with two AQP transcripts showed the significant response to the challenges. The mmAQP1a mRNAs in the liver and kidney were significantly induced by LPS or poly(I:C) injection. In contrast to expression levels of mmAQP1a transcripts, AQP3a expression was markedly elevated in some but not all tissues examined. In particular, the AQP3a transcript was significantly elevated in the liver or kidney in response to challenge. Furthermore, the significant AQP3a level after poly(I:C) stimulation was striking when compared to basal expression in the liver. Recent study has reported that hepatic AQP3 might be involved in both Kuffer cell migration and proinflammatory cytokine secretion in mammals, reflecting its involvement with immune response (Rodríguez et al. 2011). The expressions of mud loach aquaporin gene under *E. tarda* stimulus are differentially modulated during experimental period, as in the aforementioned results. The intestinal AQP1a and AQP3a transcript levels showed a significant reduction with time. Apart from serving as the site of nutrient uptake and osmoregulation, the intestine functions as a critical defense barrier to the external environment (Grosell 2011). The intestinal fluid balance in the fish as well as mammalian has been indicated to be cooperated by various factors, including hormones, intestinal contents, inflammatory factors, and feeding (Rombout et al. 2011; Zhu et al. 2016). Thus, the bacterial challenge may either directly or indirectly linked to unfavorable change of water balance in the intestine in the pathophysiological stress. In the kidney, the expression of two isoform genes was modulated in opposite directions in response to *E. tarda* challenge. When considering that the role of kidney in freshwater fish is to produce copious quantities of dilute urine, the significant fluctuation in renal AQP expression after bacterial challenge may be associated with the conditions demonstrating severe edema formation due to renal failure, as evidenced by zebrafish (Kramer-Zucker et al. 2005). Usually, the piscine liver performs not only the basically metabolic function including bile formation and excretion but also macrophage contributor (Paulsen et al. 2003; Wolf and Wolfe 2005). Accordingly, modulation of hepatic AQP3 transcript may result in disruption of physiological homeostasis, for example, bile secretory failure (Lehmann et al. 2008). Collectively, these data suggest that the alteration in AQP transcripts expression, especially AQP3a, may be involved in a significant

way in the pathophysiology of fish and should be considered in further studies.

Conclusions

In current study, we observed ubiquitous distribution of mud loach AQPs in immune-relevant as well as osmoregulatory tissues. Immune challenge-induced changes in expression pattern of AQP3 indicate that this gene has important role to respond to inflammatory disease or condition. Further investigation is needed to decipher the importance of AQPs in dealing with water homeostasis during infection and inflammation in a finer detail.

Abbreviations

AQP: Aquaporin; EST: Expressed sequence tag; hpi: Hours post injection; LPS: Lipopolysaccharide; MIP: Major intrinsic protein; NPA: Asparagine-proline-alanine; ORF: Open reading frame; poly(I:C): Polyinosinic: polycytidylic acid

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Availability of data and materials

Not applicable.

Authors' contributions

YKK and SYL carried out the molecular biology and expression assay. YKK and YKN designed the study and data evaluation and drafted the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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