

Structural and evolutionary divergence of aquaporins in parasites (Review)

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Abstract. Aquaporins are ubiquitous proteins that belong to the major intrinsic protein family. Previous studies have indicated that aquaporins are involved in multiple physiological processes in parasites, such as nutrient absorption and end product efflux, and thus, would be promising pharmacological agents in the fight against parasite infection. In the present paper, the authors analyzed the evolutionary relationship of parasitic aquaporins by re-constructing of a phylogenetic tree using neighbor-joining and maximum likelihood methods. In addition, the authors discussed the variation of the conserved functional sites impacting on the transportation of water molecules. The protein was concluded to be a potential drug target in parasites.

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1. Introduction

Parasites can be divided within a diverse phylum composed of protozoan and helminthes, and this phylum contains numerous species that can infect humans and animals. Some parasitic species can even infect plants, and have been affecting humans for many years (1). Emerging parasitic diseases in humans and animals, such as malaria, toxoplasmosis, leishmaniasis, schistosomiasis, echinococcosis, trichinellosis and cysticercosis, are global problems, which can lead to serious nutritional deficiencies, reduce animal productivity, effect acute tissue damage or even death (2-5).

Considering that parasites have diverse and complex biological and ecological lifestyles, this raises questions as to how can they maintain their fluid homeostasis under different osmotic stress *in vivo* and *in vitro*, and also how parasites regulate rapid water transport in moist and dry environments. What is interesting about these parasites are the channels and transporters (also known as permeases) in membranes with essential biological functions involving in facilitating water transport and osmoregulation, nutrient uptake, cytotoxic release and host cellular adhesion (6,7).

A significant discovery concerning channels in cell membranes awarded the Nobel Prize in Chemistry in 2003 to Peter Agre, who was famed for identifying the functions of aquaporins (AQPs). In the present paper, the authors review the amino acid residue divergence and analyze the evolutionary relationship of parasite aquaporins, and further discuss the possibility of developmental drug design against helminths based on the protein.

AQPs belong to the major intrinsic protein (MIP) family and exist in almost all living organisms including animals, plants, bacteria and viruses (8). The aquaporin family is abundant in the plasma membrane (9). Proteomics analyses indicate that aquaporins are the most plentiful proteins within the biological tegument surface of *Schistosoma mansoni* (10). The common structure of AQPs consists of 6 α -helical transmembrane domains (TM) and five connecting loops (11). The N- and C-terminal regions of AQPs, as well as B and D loops, are within the cytoplasm.

The advanced structure of AQP proteins is often compared to an hourglass, whereby the center of the pore has unique

Asn-Pro-Ala (NPA) sequence motifs in loops B and E; this is where an isolated water molecule will transiently form hydrogen bonds with two conserved Asn residues (12). The narrowest restricted site is located at the entrance of the pore mouth with a diameter of 2.8 Å, which may interact with passing solutes, bound by aromatic amino acids and a widely conserved aromatic arginine (ar/R) filter (12,13).

2. Re-construction of phylogenetic tree based on aquaporin sequences

To investigate the evolutionary relationship of MIP family proteins in parasites, the deduced polypeptides were investigated by ClustalW multiple sequence alignment software and the phylogenetic tree of parasites was re-constructed using the neighbor-joining (NJ) and maximum likelihood (ML) methods, based on the AQP sequences (Fig. 1). A total of 44 AQP sequences were selected. The most outstanding phylogenetic pattern is divided into two distinct clusters that are orthodox aquaporins and aquaglyceroporins (GLPs). The orthodox aquaporin, also named classical aquaporin, only allow the passage of water, while the aquaglyceroporins can also transport uncharged molecules like polyols, urea and metalloids besides water (14). In the phylogenetic tree, a subcellular-aquaporin group is also formed as an independent cluster, due to the unusual conserved NPA motifs (15,16).

Unlike the aquaporins in plants and mammalian animals, the protein in parasites seems to have fewer isoforms (17), and thus, the few channels tend to be multifunctional. The assembled functions in a single protein are not only due to the simple physiological structures of parasites, but also due to the probability of a self-protection strategy that parasites use to reduce excessive antigen exposure to the host-parasite interface; this avoids activating the host immune response system.

As revealed from the phylogenetic tree in the present study, GLP group can be further divided into four major GLPs sub-groups: i) Protozoan GLPs+*Homo sapiens* (Hs) AQP₇ and *Escherichia coli* (Ec) GlpF, ii) nematode GLPs + HsAQP_{3,9,10}, iii) trematode GLPs, and iv) cestode GLPs. These parasites possess at least one multi-functional aquaporin. It is noteworthy that both FgAQP₁ and FgAQP₂ were integrated into in a single branch belonging to subcellular-aquaporin group, both of which contain a mutated TAA motif in the B loop (18). FgAQPs are primarily expressed in tegumental cells and the linings of ovary and testes (18). Increased water permeability observed in *Xenopus* oocytes, but a failure to permeate glycerol and urea (18) suggests that FgAQPs may be more likely to involve osmoregulation in *Fasciola* instead of transporting solutes.

Similarly interesting is the distribution of *Toxoplasma gondii* (Tg)AQP. In the phylogenetic tree, TgAQP belongs to the water specific group that presents 47% similarity to plant tonoplast intrinsic proteins, which is a water specific channel in theory. Pavlovic-Djuranovic, Schultz and Beitz (19) reported that TgAQP is a bifunctional channel, permeating both water and glycerol, which suggests that TgAQP has obtained solute permeability following gene transfer from plant to an ancestor of *Toxoplasma*.

3. The variation of restrictions in aquaporin proteins from parasites

Aquaporin integrates into a homotetramer in the plasma membrane and each monomer is an independent functional pore (20). The central pore formed by four monomers is said to be a gas channel for CO₂ and NO (21). Arg (ar/R) constriction and NPA motifs are two major constriction regions that are responsible for aquaporin selectivity. The ar/R constriction region is the narrowest part of the water channel, and the residues are Phe56, His180, Cys189 and Arg195 in human AQP1 and Trp48, Gly191, Phe200 and Arg206 in *E. coli* GlpF. The substitution of His180, Cys189 in GlpF by Gly191 and Phe200 may result in the changed size and the increased hydrophobicity, which allow a larger molecule, such as glycerol, to pass through (22,23).

Here, the ClustalW analysis demonstrated that the most remarkable point lies in the highly conserved arginine residue (Figs. 2 and 3) following the second NPA box, except in 4 AQP sequences (TbAQP₂, TgAQP, FgAQP_{1,2}, HsAQP_{11,12}). Arginine is a polar molecule with positive charges, and serves a vital role in the transport selectivity and expulsion of proton (23). The removal of the positive charge AQP1-R195V or AQP1-H180A/R195V would result in proton leak (23). In TgAQP, the Arg204 residue mutates into valine, a similar mutation to the plant TIP subfamily (19). The unusual selectivity filter replaces Arg264 with Leu264 in TbAQP₂ and displays the ability to allow the passage of large molecules like melarsoprol and pentamidine through the channel (24). As a result, TbAQP₂ may transport larger solutes compared with TbAQP₃, and may be responsible for its susceptibility of melarsoprol and pentamidine (24). In addition, the cysteine residue serves a significant role in the ar/R restriction. Cys in AQPs is a vital residue reacting to mercurial compounds. HsAQP₁ (C189) and HsAQP₉ (C213) can be inhibited by mercuric chloride (25,26). It can be suggested that cysteines at position 204, 203 and 220 in FgAQP₁, FgAQP₂ and OvAQP₃, respectively, may also be mercury-sensitive sites and could be blocked by mercury ions.

The sequences used in the current work indicated that the functional residues were corresponding to the Froger's prediction method (27) at P₁₋₅ positions, although limited aquaporin sequences have been used (40 aquaporin sequences in total, 14 for GlpF cluster, 26 for AQP cluster). In addition, the authors previously identified some more conserved residues (data not shown), but their roles are still unclear and need to be confirmed later in detail.

4. The variation of the NPA motifs in aquaporin of parasites

NPA motifs are characteristics of MIP family members, comprising Asn-Pro-Ala (11). The Asn residue can be considered as the secondary proton filter pointing towards the inner channel, of which the side chains serve as hydrogen-bond donors, forming new hydrogen bonds with travelling water molecules (28).

As presented in the aligned sequences, NPA boxes (Figs. 2 and 3) are highly conserved in MIP family members, despite of a few variations. The variations were primarily at the second or third position in the first NPA motif, except in FgAQP_{1,2}. The NPA motif in FgAQP_{1,2} comprises TAA motif

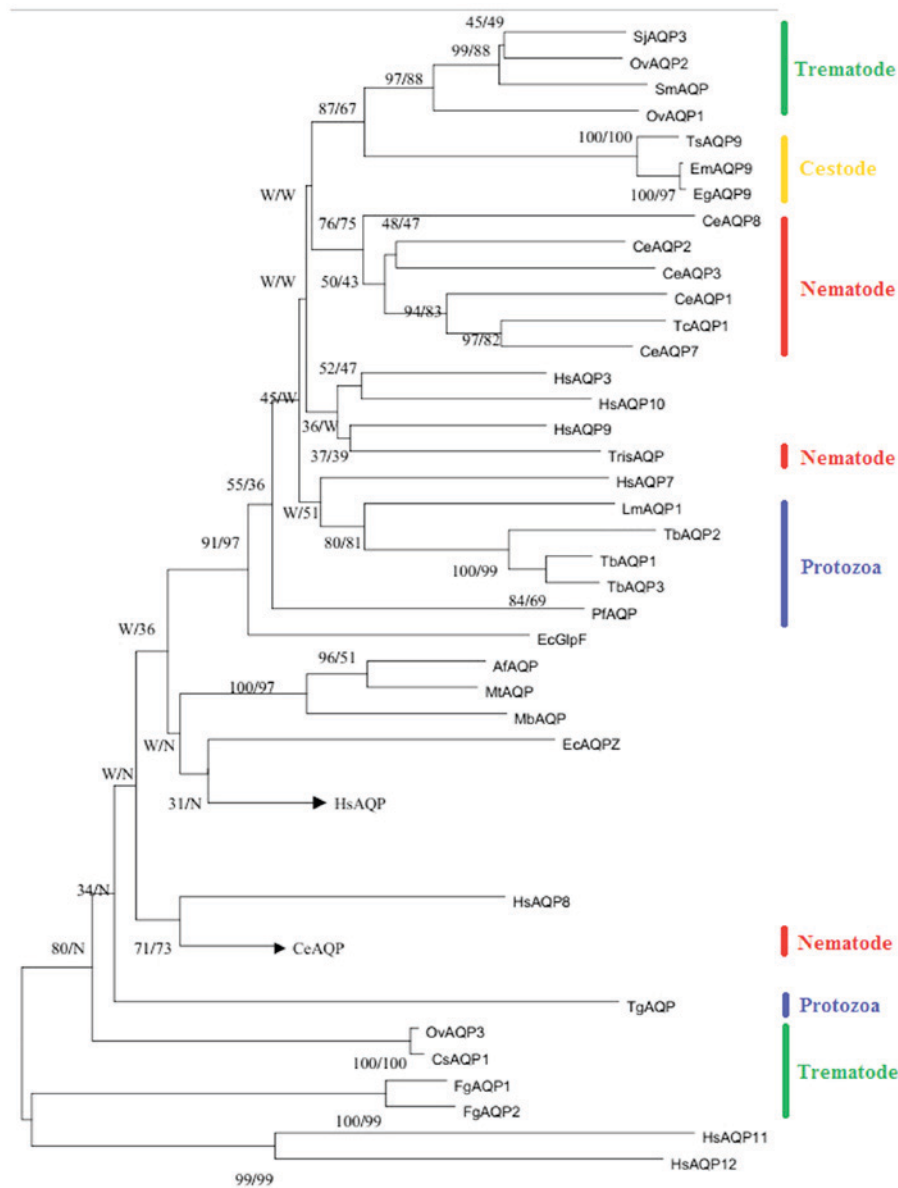


Figure 1. The phylogenetic tree re-constructed using NJ and ML methods to elucidate the evolutionary relationship between parasitic aquaporin sequences. The accession numbers for each sequence are as follows: PfaQP (AJ413249); LmAQP1 (AY567835); TbAQp1-3 (AJ697889, AJ697890, AJ697891); TgAQp (*Toxoplasma gondii*); SjaQP3 (CPRT0000005211); SmAQp (EU780065); FgAQp1-2 (HM748645, HM748644); TcAQp1 (AF067963); CeAQp1-8 (CCD66276, CAA84633, CAA22259, CAA94770, CAA94903, CCF23336, CCD66489, CCD68566); HsAQp1-12 (NP_932,766, AAB31999, CAG46822, CAG46819, NP_0,01643, CAI13303, AAH40630, CAG46824, CAH70483, Q8NBQ7, Q8IXF9); TrisAQp (unclear); EcGlpF (CDZ22687); EcAQpZ (AAC43518); EmAQp9 (CDS35949); EgAQp9 (CDS22736); TsAQp9 (000,547100); AfaQP (NP_07,0255); MbaQP (ZP_00,077803); MtaQP (AB055880); OvaQP1-3 (KF697690, KF697691, KM359766); CsAQp1 (GAA33659). The numbers indicate bootstrap values resulting from different analyses in the order NJ/ML. NJ, neighbor-joining; ML, maximum likelihood; AQP, aquaporin; Pf, *Plasmodium falciparum*; Lm, *Leishmania major*; Tb, *Trypanosoma brucei*; Tg, *Toxoplasma gondii*; Sj, *Schistosoma japonicum*; Sm, *Schistosoma mansoni*; Fg, *Fasciola gigantica*; Tc, *Toxocara canis*; Ce, *Caenorhabditis elegans*; Hs, *Homo sapiens*; Tris, *Trichinella spiralis*; Ec, *Escherichia coli*; Em, *Echinococcus multilocularis*; Eg, *Echinococcus granulosus*; Ts, *Taenia solium*; Af, *archaeoglobus fulgidus*; Mb, *Methanosarcina barkeri*; Mt, *Methanothermobacter thermoautotrophicus*; Ov, *Opisthorchis viverrini*; Cs, *Clonorchis sinensis*.

(Thr-Ala-Ala), and the similar formation is demonstrated in the *Burkholderia coenocephala* SPA at a corresponding position (29). In fewer cases, including PfaQP, TbAQp₂, CeAQp₅ and HsAQp₇, both of the two NPA motifs become distinct in a single aquaporin sequence (30,31). These mutations alter the bulk and polarity of amino acid residues, which leads to the changed hydrophilic property and the physiological roles. Replacement of Thr by Asn at the first TAA motif of FgAQp1 made its water flux increase two-fold when expressed in *Xenopus laevis* oocytes, however, this alteration does not alter the impermeability of urea and glycerol (18). In

Trypanosoma brucei, TbAQp2 contains two non-canonical NSA/NPS motifs that are scarcely expressed. Perhaps the protein is an organelle aquaporin and is expressed only under given stress conditions (30).

The altered motifs, however, do not always alter its transport properties (31). In *Plasmodium falciparum*, the classical NPA sequences were substituted with the uncommon NLA and NPS sequences in B and E loops, respectively. After the divergent motifs are converted to canonical NPA motifs, the swelling rates of the mutative protein to water and glycerol are equal with wild type PfaQP (31). An identical variation

| | | Motif | | | | | | | | | | Motif | | | | | | | | | | | | | | | | | | | | |
|----------------|----|-------|---|---|---|---|---|---|---|---|---|-------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|
| <i>OvAQP3</i> | 66 | G | P | V | S | G | A | H | V | N | P | V | V | S | - | D | L | T | G | A | S | M | N | P | A | R | S | F | G | P | A | 204 |
| <i>CeAQP5</i> | 94 | G | K | I | S | G | G | H | F | N | P | V | V | S | - | S | I | T | G | T | A | M | N | P | V | R | A | L | S | P | N | 266 |
| <i>AfAQP</i> | 75 | G | R | I | S | G | A | H | I | N | P | A | V | T | - | N | I | T | G | S | S | L | N | P | A | R | T | F | G | P | Y | 208 |
| <i>FgAQP1</i> | 77 | G | P | I | N | G | P | Q | I | T | A | A | L | S | - | K | V | T | G | C | G | M | N | P | A | A | S | L | S | A | A | 215 |
| <i>FgAQP2</i> | 77 | G | P | V | S | G | P | Q | L | T | A | A | L | S | - | K | V | T | G | C | G | L | N | P | A | S | S | L | A | G | A | 214 |
| <i>HsAQP11</i> | 91 | L | T | L | V | G | T | S | S | N | P | C | G | V | - | S | L | T | G | A | V | F | N | P | A | L | A | L | S | L | H | 209 |
| <i>HsAQP12</i> | 73 | V | T | L | D | G | A | S | A | N | P | T | V | S | - | P | F | T | S | A | F | F | N | P | A | L | A | A | S | V | T | 208 |
| <i>HsAQP1</i> | 68 | G | H | I | S | G | A | H | L | N | P | A | V | T | - | P | V | T | N | T | S | V | N | P | A | R | S | T | A | V | A | 200 |
| <i>EcAQPZ</i> | 55 | G | H | I | S | G | G | H | F | N | P | A | V | T | - | P | V | T | N | T | S | V | N | P | A | R | S | T | A | V | A | 194 |
| - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |

Figure 2. Partial aquaporin sequences (including FgAQP1-2, HsAQP11-12) were aligned to display the variations at NPA motifs and Arg position. *No variation. Fg, *Fasciola gigantica*; AQP, aquaporin; Hs, *Homo sapiens*; Ov, *Opisthorchis viverrini*; Ce, *Caenorhabditis elegans*; Af, *archaeoglobus fulgidus*; Ec, *Escherichia coli*.

| | | Motif | | | | | | | | | | Motif | | | | | | | | | | | | | | | | | | | | |
|---------------|-----|-------|---|---|---|---|---|---|---|---|---|-------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|-----|
| <i>PfAQP</i> | 63 | K | L | S | G | A | H | L | N | L | A | V | S | I | - | G | N | T | G | F | A | L | N | P | S | R | D | L | G | S | R | 201 |
| <i>TbAQP2</i> | 123 | G | I | S | G | G | H | L | N | S | A | V | T | V | - | L | A | S | P | L | A | M | N | P | S | L | D | F | G | P | R | 269 |
| <i>TgAQP</i> | 86 | D | I | S | G | A | H | F | N | P | A | V | S | C | - | A | F | A | G | A | T | M | N | P | A | V | T | F | G | I | F | 209 |
| <i>SjAQP3</i> | 87 | C | G | G | S | G | H | I | N | P | A | I | T | L | - | L | N | A | G | A | A | L | N | P | A | R | D | L | G | P | R | 233 |
| <i>SmAQP</i> | 84 | Q | C | G | S | G | H | C | N | P | A | V | T | L | - | L | N | A | G | A | A | L | N | P | A | R | D | L | G | P | R | 230 |
| <i>TcAQP1</i> | 88 | K | T | S | G | G | H | L | N | P | A | I | S | L | - | M | N | V | G | Y | P | I | N | P | A | R | D | F | A | P | R | 234 |
| <i>CeAQP1</i> | 86 | R | M | S | G | G | H | L | N | P | A | V | S | L | - | M | N | I | G | Y | P | I | N | P | A | R | D | L | G | P | R | 232 |
| <i>HsAQP7</i> | 87 | R | I | S | G | A | H | M | N | A | A | V | T | F | - | A | I | T | D | Q | E | N | N | P | A | L | P | G | T | E | A | 203 |
| <i>HsAQP9</i> | 77 | G | V | S | G | G | H | I | N | P | A | V | S | L | - | L | N | S | G | C | A | M | N | P | A | R | D | L | S | P | R | 224 |
| <i>EcGlpF</i> | 61 | G | V | S | G | A | H | L | N | P | A | V | T | I | - | P | L | T | G | F | A | M | N | P | A | R | D | F | G | P | K | 211 |
| - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | |

Figure 3. Partial GlpFs sequences were aligned to display the variations at NPA motifs and Arg position. *no variation. Pf, *Plasmodium falciparum*; Tb, *Trypanosoma brucei*; Tg, *Toxoplasma gondii*; Sj, *Schistosoma japonicum*; Sm, *Schistosoma mansoni*; Tc, *Toxocara canis*; Ce, *Caenorhabditis elegans*; Hs, *Homo sapiens*; Ec, *Escherichia coli*.

at the first NPA (changed into NPV) motif was identified in *OvAQP₃* and *CsAQP₁* (32). The *OvAQP₃* was predicted to be a similar advanced structure to mammalian *AQP₁*, the specific water channel. However, *OvAQP₃* can transport glycerol and urea, but not only water molecules, which suggests that sequence-based aquaporin classification do not completely reveal its real function in parasites (32).

5. Potential drug targets in parasites: Aquaporins

Aquaporins can regulate multiple significant physiological roles in living organisms, which means that they are regarded as prime candidates for pharmacological intervention in human diseases (33). The numbers of aquaporin-encoding genes are varied in parasites, for instance, there are five genes in *Leishmania major* and one gene in *P. falciparum* (31,34). AQPs are desired as novel targets for antiparasitic drugs (35,36).

Aquaglyceroporins isolated from *Leishmania major* (Lm) *AQP1* and *Schistosoma mansoni* (Sm)*AQP* are physiological water channels. They can act as conduit for parasite-killing drugs and increase the accumulation of metalloids, such as As(III) and Sb(III) in *L. major* and Potassium Antimonyl Tartrate in *S. mansoni* (36,37). A previous study demonstrated that, following silencing *SmAQP* using specific

siRNA, the treated *Schistosoma* exhibits more resistance to PAT (36). Proteomic analysis of the *Schistosoma* tegument membranes presented a single AQP homologue (38), which exhibits 31-36% sequence identity with human *AQP 3,7,9* and *10*. In addition, *SmAQP* may also act as a channel for glycolytic end-products to avoid the accumulation of toxins (39). The Arg residue located inside the vestibule of the channel may also severely affect the metalloid sensitivity (40). For instance, alteration of Arg230 in *LmAQP1* to Ala or Lys presented a negligible low level transport of metalloid in cells, and therefore appeared more resistant to As(III) and Sb(III) than cells expressing wild type *LmAQP1* (41). The side chain of the Ala residue at the position of 163 in *LmAQP1* may serve a role in drug resistance because of a steric hindrance effect (42). The Thr164 substituted by Cys in *LmAQP1* altered the mercurial sensitivity that can be blocked by $HgCl_2$ (42). These studies suggested that aquaporin in parasites possess transport property for drugs and, therefore, may have potential access to be a viable candidate for therapeutic targets.

6. Conclusions and perspectives

Since the discovery of the first aquaporin, the protein has attracted considerable interests in the development of new

pharmacological agents for the treatment of parasitological diseases, due to their potential structure and functions. In general, aquaporins are conserved between species during the evolutionary process, especially at the transmembrane regions and functional sites (e.g., NPA and ar/R). In parasitic aquaporins, variations seem to have differential effects for different aquaporin, but not always altering the selectivity of the aquaporin. As the common component of the parasite-host interface, aquaporins possess great advantages of being new chemotherapeutic targets. However, few helminthic aquaporins have been well studied.

Some compounds, such as mercuric chloride, can act as aquaporin inhibitors, interdicting the ability of the protein to transport solutes (43). Some aquaporin modulators are promising agents for the treatment of human disorders (33). Furthermore, the elucidation of 3D structures and more crystallographic information of aquaporins will increase our understanding of drug design. However, much requires elucidation in order to develop the potential applications of parasitic aquaporins. The development of mammalian aquaporins would be provided references for the studies on parasitic aquaporins. In summary, aquaporins are an ancient protein with the ability of transportation of water molecules and nutrients, osmoregulation, invasion and drug resistance. More studies on parasitic aquaporins are required.

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