

Aquaporin Water Channels and Brain Edema

MARIOS C. PAPADOPOULOS, M.D., F.R.C.S.^{1,*},

SANJEEV KRISHNA, PH.D., F.R.C.P.², AND A. S. VERKMAN, M.D., PH.D.¹

Abstract

Brain edema accounts for much of the morbidity and mortality associated with common neurological conditions such as head trauma, brain tumors, stroke and liver failure. Treatment options are limited to osmotic agents such as mannitol, surgical decompression, and other maneuvers, none of which correct the molecular-level mechanisms responsible for brain swelling. Recent data suggest that aquaporin (AQP) water-transporting proteins may provide a key route for water movement in the brain. AQP1 is expressed in choroid plexus and probably facilitates cerebrospinal fluid secretion. AQP4 is expressed in astrocyte foot processes near capillaries and in ependymal cells lining the ventricles — key sites for water movement between the cellular, vascular, and ventricular compartments. AQP4 expression is markedly altered in experimental models of brain injury and swelling, and transgenic mice lacking AQP4 are partially protected from brain swelling in response to acute hyponatremia and ischemic stroke. Aquaporins and regulators of brain aquaporin expression are thus potential targets for discovery of compounds for treatment of brain swelling.

Key Words: Brain swelling, cytotoxic edema, vasogenic edema, water transport, water permeability, stroke, tumor, encephalopathy, transgenic mice.

Brain Edema as a Clinical Problem

EDEMA IS DERIVED FROM the Greek *οίδημα* for “swelling.” Brain edema is produced when fluid accumulates in the brain parenchyma (Fig. 1). It is seen in primary brain pathologies such as stroke, head injury, brain tumor, and brain abscess. Brain edema is also seen in globally important systemic infections that primarily involve the brain (such as childhood cerebral malaria, African trypanosomiasis and meningitis) and in conditions that effect the brain indirectly (e.g., sepsis, hyponatremia, and liver and



Fig 1. Contrast-enhanced head CT scan of a patient with an abscess in the right hemisphere. The abscess is surrounded by a hypodense area (black arrows) indicating edematous brain. Note that the edema (vasogenic) predominantly involves the white matter.

¹Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco CA; and ²Department of Infectious Diseases, St. George's Hospital Medical School, London, UK.

*Present address: Department of Neurosurgery, Atkinson Morley's Hospital, Copse Hill, London SW20 0NE UK.

Address all correspondence to Alan S. Verkman, M.D., Ph.D., Cardiovascular Research Institute, 1246 Health Sciences East Tower, Box 0521, University of California at San Francisco, San Francisco, CA 94143-0521.

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kidney failure) (1–6). Brain swelling increases intracranial pressure (ICP), which impairs vas-

cular perfusion and can lead to brain ischemia, herniation and death.

Treatment options for brain edema include osmotic diuretics, corticosteroids, maintenance of normocapnia, surgical excision of the underlying lesion, barbiturate coma, hypothermia and decompressive craniectomy. For critically ill patients, invasive monitoring of ICP and cerebral perfusion pressure is done to optimize therapy (7). However, many of these therapies to reduce brain swelling were introduced early in the 20th century and their efficacy is limited (8, 9). The paucity of effective drugs to be used against brain edema reflects, among other things, the incomplete understanding of the molecular mechanisms involved in the formation and resolution of brain edema.

Mechanisms of Edema

The volume of the intracranial cavity containing the brain parenchyma, the cerebrospinal fluid and blood is about 1200–1400 mL in humans (Fig. 2A). Exchange of fluid between these compartments occurs at the blood-brain barrier, ventricular ependyma, choroid plexus, and arachnoid granulations. In addition, up to 30 mL of water is produced daily from glucose metabolism. Osmotic gradients and hydrostatic pressure differences are the forces that drive water movement between the intracellular, interstitial, ventricular cerebrospinal fluid (CSF) and vascular (blood) compartments in the brain (Fig. 2A).

Brain edema is the accumulation of water in the brain parenchyma. Because the adult

skull is mechanically rigid, an increase in brain parenchymal volume results in displacement of fluid from the low-pressure (~10 mm Hg in man) CSF, low-pressure (~10 mm Hg) venous, and high-pressure (~105 mm Hg, mean arterial pressure) arterial compartments (Monro-Kellie doctrine). The ability of the intracranial contents to resist a rise in intracranial pressure (ICP) as the brain parenchyma volume increases is largely due to the capacity of the CSF and venous compartments to contract (Fig. 2B).

Igor Klatzo classified brain edema as vasogenic or cytotoxic (10). Vasogenic edema occurs when the blood-brain barrier becomes leaky, permitting the entry of plasma fluid into the brain parenchyma (3, 5). Brain tumor edema is an example of vasogenic edema. Vasogenic edema fluid is extracellular and accumulates primarily in white matter, because resistance to fluid flow is less in white than in grey matter. Tight junctions in microvascular endothelia open in vasogenic edema. The recent discovery of tight-junction-associated proteins (occludin, claudin, JAM, ZO-1, ZO-2, ZO-3) may provide some clues to the molecular mechanisms involved in the breakdown of the blood-brain barrier. For example, glioblastomas and metastatic brain carcinomas, which can be associated with marked brain edema, show abnormal expression of endothelial cell occludin (11), claudin-1 (12), claudin-5 (12) and ZO-1 (13).

Cytotoxic edema consists mainly of intracellular fluid accumulation that occurs during water intoxication and anoxia-generating conditions such as stroke, trauma and hypoxia. Cytotoxic edema also results as a direct consequence of infections such as cerebral malaria, which causes microvascular obstruction due to adherence of *Plasmodium falciparum*-infected erythrocytes to cerebral capillaries (1, 2). It is believed that the astrocyte is the major cell type showing swelling after ischemia and trauma (14). Astrocyte swelling may be an important early event predisposing the brain to further damage, because of the impairment of protective homeostatic mechanisms. Astrocytes *in vitro* show regulatory volume decrease in response to hypoosmotic media, with loss of intracellular ions (mainly K^+ and Cl^-) and amino acids (including the excitotoxic amino acid glutamate) (5, 14). However, it is not clear whether these astrocyte volume regulatory mechanisms occur during neuronal damage *in vivo*.

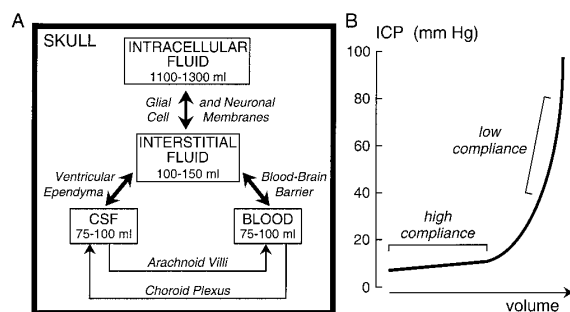


Fig 2. A. Schematic of intracranial fluid compartments (in boxes) and the directions of water movement between them (arrows). Arrows are labeled with barriers separating indicated compartments. B. Intracranial pressure (ICP) as a function of the volume of an expanding intracranial lesion. As the lesion expands there are compensatory mechanisms (reduction in CSF and cerebral blood volumes) that maintain a normal ICP (high compliance). Further volume expansion produces large increases in ICP (low compliance).

Aquaporin Water Channels

Physiologists recognized long ago that water crosses the plasma membrane of many cell types (e.g., erythrocytes, kidney tubules) substantially faster than can be explained by simple diffusion. Over the past decade a family of related water-transporting proteins, called aquaporins (AQP), has been identified (15, 16). At least ten aquaporin water channels have been cloned from mammals and many more from amphibians, plants, yeast, bacteria and other lower organisms. The aquaporins are small (~30 kDa) hydrophobic proteins that assemble in membranes as tetramers. Each monomer, consisting of six membrane-spanning tilted α -helical domains with cytoplasmically oriented amino and carboxy-termini, contains a distinct water pore (17, 18). The aquaporins can be classified functionally into two groups. AQP1, AQP2, AQP4, AQP5 and AQP8 are mainly permeable to water. AQP3, AQP6, AQP7 and AQP9 are also water permeable, but in addition have significant permeability to small solutes such as glycerol (AQP3, AQP7 [aquaglyceroporins]) and larger neutral solutes (AQP9), and possibly under some conditions, to ions (AQP6).

The sites of aquaporin expression suggest a role in renal water reabsorption, CSF dynamics, aqueous fluid dynamics, glandular secretion, and other physiological processes. Humans with mutations in AQP2, the vasopressin-regulated water channel in the kidney collecting ducts, have the rare disease, hereditary nephrogenic diabetes insipidus, which manifests as severe polyuria (19). Unfortunately, aquaporin inhibitors suitable for use *in vivo* do not exist, which has made it difficult to define the role of aquaporins in organ physiology. However, recent phenotype studies in transgenic mice lacking AQPs 1–5 have been very informative (20, 21). For example, mice lacking AQP1, AQP2 or AQP3 have defective urinary concentrating ability (22–27), mice lacking AQP4 have impaired hearing (28), and mice lacking AQP5 have impaired secretion by salivary gland (29) and airway submucosal glands (30). The phenotype studies suggest that aquaporins are important for rapid, near-isosmolar fluid transport, as occurs in kidney proximal tubule and salivary gland, and for water transport when it is driven by established osmotic gradients, as in kidney collecting ducts. The phenotype studies have also shown that the tissue-specific expression of an aquaporin does not indicate physio-

logical significance — for example, deletion of AQP4 in skeletal muscle and gastric parietal cells does not impair skeletal muscle function (31) or gastric acid production (32). The aquaporin null mice provide a useful tool for defining the role of aquaporins in fluid balance in the central nervous system.

Aquaporin Expression in the Brain

AQP1 and AQP4 are expressed strongly in the brain. In normal rat brain, AQP1 transcript and protein expression is restricted to the ventricular-facing surface of choroid plexus (Fig. 3A) (33, 34). AQP1 was initially cloned from

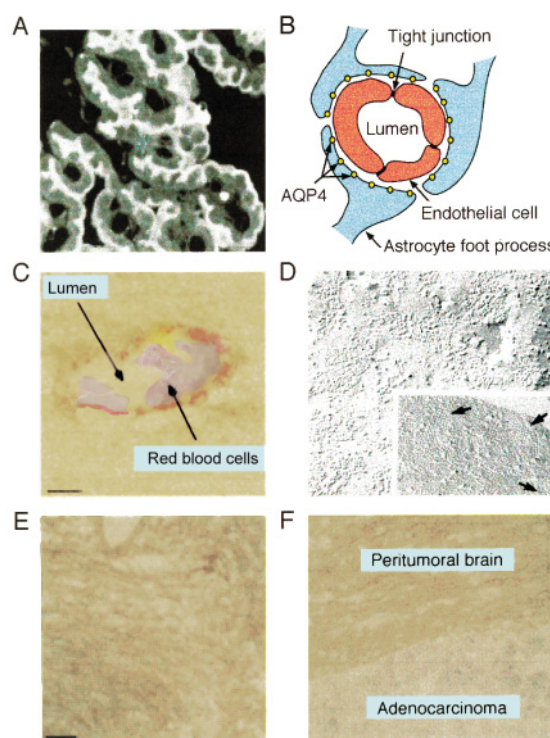


Fig 3. A. Immunofluorescence showing AQP1 protein expression in the apical surface of choroid plexus cells in rat. B. Diagram of a brain microvessel in cross section. Endothelial cells (red) are joined by tight junctions (black) and surrounded by astrocyte foot processes (blue). AQP4 water channels (circles) are expressed at the astrocyte foot processes. C. In normal human brain parenchyma AQP4 immunoreactivity (brown) is seen mainly around the microvessels. Bar: 10 μ m. D. Freeze-fracture electron micrograph (E-face) of rat brain showing AQP4 square arrays in an astrocyte foot process. Inset shows arrays in P-face micrograph (arrows). E. Human glioblastoma multiforme immunostained for AQP4. AQP4 expression (brown) is not restricted to the perimicrovessel region, but is massively upregulated throughout the specimen. Bar: 10 μ m. F. Metastatic adenocarcinoma in human brain showing marked AQP4 immunoreactivity (brown) in peritumoral brain. Bar: 10 μ m.

human erythrocytes (35) and shown by several laboratories in heterologous expression and reconstitution studies to function as a water-selective transporter. It has been proposed that transport of ions (36) and carbon dioxide (37) through AQP1 occurs under some conditions, though such transport, if it occurs, is probably not of physiological significance (38). AQP1 protein has been visualized directly by freeze-fracture electron microscopy, as small intramembrane particles with a tetrameric structure (39). The expression of AQP1 in choroid plexus, taken together with the paradigms established from the mouse phenotype studies of non-neural tissues mentioned above, suggests that AQP1 plays a role in CSF secretion.

AQP4 is expressed in astrocyte foot processes near blood vessels in rat (40, 41) and human (42) brain (Figs. 3B, 3C), as well as in ependymal and pial surfaces in contact with CSF (41, 43, 44). This localization suggests that AQP4 plays a critical role in brain water balance. AQP4 was initially cloned from rat lung (45), and subsequently isoforms from rat brain (46) and orthologues from human (47) and mouse (48) were sequenced. AQP4 appears to be selective for the passage of water, and is unique among the aquaporins in its exceptionally high intrinsic water permeability (49) and its assembly in membranes in regular square arrays called orthogonal arrays of particles (OAPs) (Fig. 3D). The involvement of AQP4 in OAP formation was originally proposed because of the expression of AQP4 in tissues known to contain OAPs (44), and it was later proven in heterologously expressing cell cultures (50) and as a result of the absence of OAPs in AQP4 null mice (51). Immunogold-label freeze-fracture electron microscopy confirmed the presence of AQP4 in OAPs in the brain (40, 41).

Other aquaporins may also be expressed in the brain, though the evidence is less compelling. In rats (52) and mice (53), AQP9 protein has been detected in cells lining the cerebral ventricles, including ependymal cells and tanyocytes, and possibly at low levels in astrocytes. There may be other as yet unidentified aquaporins in the brain, and some non-aquaporin proteins, such as glucose, urea and ion transporters, may transport small amounts of water.

Indirect Evidence for a Role of Aquaporins in Brain Edema

A number of studies indicate that the expression of aquaporins in the brain is sensitive

to brain injury, swelling and other experimental maneuvers. In rodents, AQP4 expression in astrocytes is upregulated in response to cerebral edema caused by brain injury (54, 55), focal brain ischemia (56) and hyponatremia (57). AQP4 expression is also upregulated in edematous human tumors (Figs. 3E, 3F), where AQP4 loses its polarity and redistributes throughout the astrocytes. After middle cerebral artery occlusion in rats, the increase in AQP4 transcript expression parallels brain edema, as monitored by magnetic resonance imaging (56). In rat models of brain injury (54), increased AQP4 expression occurs in astrocytes at sites where the blood-brain barrier is disrupted. Increased AQP4 expression thus appears to occur in edematous brain regardless of the underlying cause of edema. If AQP4 contributes to brain edema, then its upregulation may be a maladaptive response, as is the case for upregulation of AQP2 in the kidney, in some fluid-retaining states.

Expression of AQP1 protein has been reported in endothelial cells in rat models of glioblastoma and metastatic brain cancer (58). A microarray study of differential gene expression has also found increased AQP1 transcript expression in glioblastoma, which has been confirmed by AQP1 immunohistochemistry on tumor samples (59). AQP1 expression in microvessels of neoplastic brain may contribute to increased blood-brain barrier water permeability in aggressive brain tumors. In normal brain, AQP1 expression in choroid plexus is downregulated in mice exposed to hypogravity during spaceflight (34), which may be a compensatory response to changes in CSF distribution. It is not known whether AQP1 expression in choroid plexus is altered in response to elevated ICP.

After transient focal brain ischemia in mice, AQP9 protein, which is normally only weakly expressed in mouse astrocytes, appears to be upregulated in astrocytes in peri-infarct areas (53). Since AQP9 is permeable to water and lactate, it was proposed that AQP9 may be involved in reperfusion edema associated with lactic acidosis. However, due to the lack of aquaporin channel blockers it is unclear whether the increased expression of AQP4, AQP1 and AQP9 in edematous brain is functionally important or represents an epiphenomenon.

Regulation of Aquaporin Expression

Little is known about the molecular mechanisms involved in regulated aquaporin expression in brain. Developmental studies indicate

that AQP4 protein expression in rat brain is very low before birth and increases steadily over the first two weeks *post partum* (60). AQP4 expression in astrocytes appears to be cell cycle-dependent and only expressed in the G0/G1 phase (61). The human AQP4 gene, located on chromosome 18, encodes proteins of 31 and 34 kDa that are produced by alternative splicing and separate promoters (62). Both isoforms are expressed in the brain, but the smaller isoform is the one mainly found in other tissues. The significance of the different AQP4 isoforms is unclear.

Normal brain astrocytes, which are in contact with endothelial cells and neurons, selectively express AQP4 in astrocyte foot processes adjacent to endothelial cells. It is thus possible that the polarized AQP4 expression in astrocytes may be regulated by factors released by neurons and/or endothelial cells. This hypothesis is supported by the observation that AQP4 expression is not polarized in reactive (42) and cultured (63) astrocytes, which have no contact with endothelial cells or neurons. Protein kinase C has been shown to mildly reduce AQP4 water-transporting activity (64) and transcript expression (65). However, no molecular-level information is available about other potential regulators of AQP4 expression.

AQP1 is expressed in endothelial cells of aggressive brain tumors, but not in normal brain endothelium (58, 59). AQP1 expression in endothelial cells might be induced by vasogenic substances, such as vascular endothelial growth factor and scatter factor/hepatocyte growth factor, which are released by tumor cells. The AQP1 promoter, sequenced from erythroleukemia cells (66), contains glucocorticoid response elements, raising the possibility that the antiedema action of corticosteroids in brain tumors may be mediated partly by changes in AQP1 expression in tumor vasculature.

Direct Evidence for a Role of AQP4 in Brain Edema

Recently, the hypothesis was tested that AQP4 plays a role in the accumulation of brain water in response to two established neurological insults: acute water intoxication (producing hyponatremia) and ischemic stroke (producing brain edema by a combination of vasogenic and cytotoxic factors) (67). Experiments were done on AQP4 null mice, which are grossly phenotypically normal and do not manifest neurological abnormalities, altered blood-brain barrier

properties, or impaired osmoregulation (67, 68). Brains from AQP4 null mice show reduced osmotic water permeability, as measured in isolated membrane vesicles (68) and brain slices (69). AQP4 deletion in mice conferred remarkable protection from brain edema. The survival of AQP4 null mice after water intoxication was greatly improved (Fig. 4A), which corresponded to significantly reduced brain swelling, particularly in astrocytic foot processes. In addition, at 24 hours after brain ischemia produced by permanent middle cerebral artery occlusion, there was improved clinical outcome and much less brain swelling (Fig. 4B).

These experiments provided the first direct evidence that AQP4 is important in the formation of brain edema. It has been proposed that AQP4 is a potential target for drug discovery (70). AQP4 inhibitors might slow the accumulation of

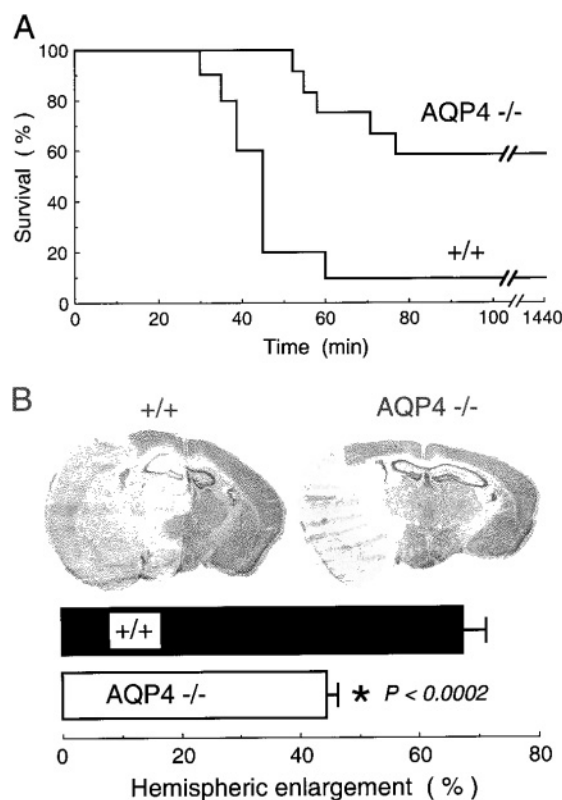


Fig 4. Reduced brain edema in AQP4 null mice after acute water intoxication and ischemic stroke. **A.** Survival of wild-type vs. AQP4 knockout mice after acute water intoxication produced by intraperitoneal water injection. **B.** (top) Brain sections of mice at 24 hours after ischemic stroke produced by permanent middle cerebral artery occlusion. Note midline shift and marked edema in brain from wild-type mice. (bottom) Average hemispheric enlargement expressed as a percentage determined by image analysis of brain sections. (Adapted, with permission, from ref. 67).

brain water, thereby reducing the morbidity and mortality of patients with neurological disorders.

Future Directions

Additional direct evidence supporting the involvement of aquaporins in brain edema is needed; in the immediate future, such evidence is likely to come from studies using aquaporin null mice. The postulated role of AQP1 in CSF production requires direct experimental verification. Although an important role for AQP4 in brain edema caused by water intoxication and focal brain ischemia has been demonstrated (67), the role of AQP4 in brain edema resulting from head injury, brain tumor and peripheral organ failure has not yet been explored. Kinetic measurements are needed to determine whether AQP4 deletion exacerbates brain edema early following head injury, and perhaps impairs the reabsorption of brain water later. Aquaporin inhibitors, which are likely to be identified by high-throughput screening or other approaches, may thus be indicated in the early phases of brain swelling. Finally, further work is needed to obtain molecular-level information about mechanisms involved in the regulation of brain aquaporin expression in tumor and other edema-inducing conditions.

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References

- Newton CW, Krishna S. Severe falciparum malaria in children: Current understanding of its pathophysiology and supportive treatment. *Pharmacol Ther* 1998; 79:1–53.
- Newton CRJC, Peshu N, Kendall B, et al. Brain swelling and ischaemia in Kenyans with cerebral malaria. *Arch Dis Child* 1994; 70:281–287.
- Papadopoulos MC, Saadoun S, Davies DC, Bell BA. Emerging molecular mechanisms of brain tumour oedema. *Br J Neurosurg* 2001; 15:101–108.
- Papadopoulos MC, Davies DC, Moss RF, et al. Pathogenesis of septic encephalopathy: a review. *Crit Care Med* 2000; 28:3019–3024.
- Kimelberg HK. Current concepts of brain edema. *J Neurosci* 1995; 83:1051–1059.
- Blei AT, Larsen FS. Pathophysiology of cerebral edema in fulminant hepatic failure. *J Hepatol* 1999; 31:771–776.
- Prough DS, Lang J. Therapy of patients with head injuries: key parameters for management. *J Trauma* 1997; 42:S10–S18.
- Roberts I, Schierhout G, Alderson P. Absence of evidence for the effectiveness of five interventions routinely used in the intensive care management of severe head injury: a systematic review. *J Neurol Neurosurg Psychiatry* 1998; 65:729–733.
- Rosomoff HL, Kochanek PM, Clark R, et al. Resuscitation from severe brain trauma. *Crit Care Med* 1996; 24:S48–S56.
- Klatzo I. Neuropathological aspects of brain edema. *J Neuropathol Exp Neurol* 1967; 26:1–14.
- Papadopoulos MC, Saadoun S, Woodrow CJ, et al. Occludin expression in microvessels of neoplastic and non-neoplastic human brain. *Neuropathol Appl Neurobiol* 2001; 27:384–395.
- Liebner S, Fischmann A, Rascher G, et al. Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme. *Acta Neuropathol (Berl)* 2001; 100:323–331.
- Sawada T, Kato Y, Kobayashi M, Takekawa Y. Immunohistochemical study of tight junction-related protein in neovasculation in astrocytic tumor. *Brain Tumor Pathol* 2000; 17:1–6.
- Kimelberg HK. Astrocytic edema in CNS trauma. *J Neurosci* 1992; 9:S71–S81.
- King LS, Agre P. Pathophysiology of the aquaporin water channels. *Annu Rev Physiol* 1996; 58:619–648.
- Verkman AS, Mitra AK. Structure and function of aquaporin water channels. *Am J Physiol* 2000; 278:F13–F28.
- Cheng A, Van Hoek AN, Yeager M, et al. Three-dimensional organization of a human water channel. *Nature* 1997; 387:627–630.
- Walz T, Hirai T, Murata K, et al. The three-dimensional structure of aquaporin-1. *Nature* 1997; 387:624–627.
- Deen PM, Verkijk MA, Knoers NV, et al. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science* 1994; 264:92–95.
- Verkman AS, Yang B, Song Y, et al. Role of water channels in fluid transport studied by phenotype analysis of aquaporin knockout mice. *Exp Physiol* 2000; 85:233S–241S.
- Verkman AS. Lessons from renal phenotype of aquaporin null mice. *Curr Opin Nephrol Hypertens* 2001; 9:517–522.
- Chou CL, Knepper MA, Van Hoek AN, et al. Reduced water permeability and altered ultrastructure in thin descending limb of Henle in aquaporin-1 null mice. *J Clin Invest* 1999; 103:491–496.
- Ma T, Song Y, Yang B, et al. Nephrogenic diabetes insipidus in mice deficient in aquaporin-3 water channels. *Proc Natl Acad Sci U S A* 2000; 97:4386–4391.
- Ma T, Yang B, Gillespie A, et al. Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. *J Biol Chem* 1998; 273:4296–4299.
- Yang B, Gillespie A, Carlson EJ, et al. Early neonatal mortality in a transgenic AQP2 knock-in model of nephrogenic diabetes insipidus. *J Biol Chem* 2001; 276:2775–2779.
- Pallone TL, Edwards A, Ma T, et al. Requirement of aquaporin-1 for NaCl driven water transport across descending vasa recta. *J Clin Invest* 2000; 105:215–222.
- Schnermann J, Chou CL, Ma T, et al. Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc Natl Acad Sci U S A* 1998; 95:9660–9664.
- Li J, Verkman AS. Impaired hearing in mice lacking aquaporin-4 water channels. *J Biol Chem* 2001; 276:31233–31237.
- Ma T, Song Y, Gillespie A, et al. Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J Biol Chem* 1999; 274:20071–20074.
- Song Y, Verkman AS. Aquaporin-5 dependent fluid secretion in airway submucosal glands. *J Biol Chem* 2001; 276:41288–41292.

31. Yang B, Verbavatz JM, Song Y, et al. Skeletal muscle function and water transport in aquaporin-4 deficient mice. *Am J Physiol* 2000; 278:C1008–C1115.
32. Wang KS, Komar AR, Ma T, et al. Gastric acid secretion in aquaporin-4 knockout mice. *Am J Physiol* 2000; 279:G448–G453.
33. Hasegawa H, Zhang R, Dohrman A, Verkman AS. Tissue-specific expression of mRNA encoding the rat kidney water channel CHIP28k by in situ hybridization. *Am J Physiol* 1993; 264:C237–C245.
34. Maseguin C, Corcoran M, Daunton NG, et al. Altered gravity downregulates aquaporin-1 expression in choroid plexus. *J Appl Physiol* 2000; 88:843–850.
35. Preston GM, Agre P. Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: Member of an ancient channel family. *Proc Natl Acad Sci U S A* 1991; 88:11110–11114.
36. Saparov SM, Kozono D, Rothe U, et al. Water and ion permeation of aquaporin-1 in planar lipid bilayers. Major differences in structural determinants and stoichiometry. *J Biol Chem* 2001; 276:31515–31520.
37. Nakhoul NL, Davies BA, Romero MF, Boron WF. Effect of expressing the water channel aquaporin-1 on the CO₂ permeability of *Xenopus* oocytes. *Am J Physiol* 1998; 274:C543–C548.
38. Yang B, Fukuda N, van Hoek AN, et al. Carbon dioxide permeability of aquaporin-1 measured in erythrocytes and lung of aquaporin-1 null mice and in reconstituted proteoliposomes. *J Biol Chem* 2000; 275:2686–2692.
39. Verbavatz JM, Brown D, Sabolic I, et al. Tetrameric assembly of CHIP28 water channels in liposomes and cell membranes. A freeze-fracture study. *J Cell Biol* 1993; 123:605–618.
40. Nielsen S, Nagelhaus EA, Amiry-Moghaddam M, et al. Specialized membrane domains for water transport in glial cells: high resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J Neurosci* 1997; 17:171–180.
41. Rash JE, Yasumura T, Hudson CS, et al. Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *Proc Natl Acad Sci U S A* 1998; 95:11981–11986.
42. Saadoun S, Papadopoulos MC, Davies DC, et al. Oedematous human brain tumours have increased aquaporin-4 expression. *J Neurol Neurosurg Psychiatry* 2002; In press.
43. Frigeri A, Gropper MA, Turck CW, Verkman AS. Immunolocalization of the mercurial-insensitive water channel and glycerol intrinsic protein in epithelial cell plasma membranes. *Proc Natl Acad Sci U S A* 1995; 92:4328–4331.
44. Frigeri A, Gropper MA, Umenishi F, et al. Localization of MIWC and GLIP water channel homologs in neuromuscular, epithelial and glandular tissues. *J Cell Sci* 1995; 108:2993–3002.
45. Hasegawa H, Ma T, Skach W, et al. Molecular cloning of a mercurial-insensitive water channel expression in selected water-transporting tissues. *J Biol Chem* 1994; 269:5497–5500.46. Jung JS, Bhat RV, Preston GM, et al. Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. *Proc Natl Acad Sci U S A* 1994; 91:13052–13056.
47. Yang B, Ma T, Verkman AS. cDNA cloning, gene organisation and chromosomal localization of a human mercurial insensitive water channel. *J Biol Chem* 1995; 270:22907–22913.
48. Ma T, Yang B, Verkman AS. Gene structure, cDNA cloning, and expression of a mouse mercurial insensitive water channel. *Genomics* 1996; 33:382–388.
49. Yang B, Verkman AS. Water and glycerol permeability of aquaporins 1-5 and MIP determined quantitatively by expression of epitope-tagged constructs. *J Biol Chem* 1997; 272:20782–20786.
50. Yang B, Brown D, Verkman AS. The mercurial insensitive water channel (AQP-4) forms orthogonal arrays in stably transfected Chinese hamster ovary cells. *J Biol Chem* 1996; 271:4577–4580.
51. Verbavatz JM, Ma T, Gobin R, Verkman AS. Absence of orthogonal arrays in kidney, brain and muscle from transgenic knockout mice lacking water channel aquaporin-4. *J Cell Sci* 1997; 110:2855–2860.
52. Elkjaer M-L, Vajda Z, Nejsum LN, et al. Immunolocalization of AQP9 in liver, epididymis, testis, spleen, and brain. *Biochem Biophys Res Commun* 2000; 276:1118–1128.
53. Badaut J, Hirt L, Granziera C, et al. Astrocyte-specific expression of aquaporin-9 in mouse brain is increased after transient focal cerebral ischemia. *J Cereb Blood Flow Metab* 2001; 21:477–482.
54. Vizuete ML, Venero JL, Vargas C, et al. Differential upregulation of aquaporin-4 mRNA expression in reactive astrocytes after brain injury: potential role in brain edema. *Neurobiol Dis* 1999; 6:245–258.
55. Ke C, Poon WS, Ng HK, et al. Heterogeneous responses of aquaporin-4 in edema formation in a replicated severe traumatic brain injury model in rats. *Neurosci Lett* 2001; 301:21–24.
56. Taniguchi M, Yamashita T, Kumura E, et al. Induction of aquaporin-4 water channel mRNA after focal cerebral ischemia in rat. *Brain Res Mol Brain Res* 2000; 78:131–137.
57. Vajda Z, Promeneur D, Doczi T, et al. Increased aquaporin-4 immunoreactivity in rat brain in response to systemic hyponatremia. *Biochem Biophys Res Commun* 2000; 13:495–503.
58. Endo M, Jain RK, Witwer B, Brown D. Water channel (Aquaporin 1) expression and distribution in mammary carcinomas and glioblastomas. *Microvas Res* 1999; 58:89–98.
59. Markert JM, Fuller CM, Gillespie GY, et al. Differential gene expression profiling in human brain tumors. *Physiol Genomics* 2001; 5:21–33.
60. Wen H, Nagelhaus EA, Amiry-Moghaddam M, Agre et al. Ontogeny of water transport in rat brain: postnatal expression of the aquaporin-4 water channel. *Eur J Neurosci* 1999; 11:935–945.
61. Yoneda K, Yamamoto N, Asai K, et al. Regulation of aquaporin-4 expression in astrocytes. *Mol Brain Res* 2001; 89:94–102.
62. Umenishi F, Verkman AS. Isolation and functional analysis of alternative promoters in the human aquaporin-4 water channel gene. *Genomics* 1998; 50:373–377.
63. Nicchia GP, Frigeri A, Liuzzi GM, et al. Aquaporin-4-containing astrocytes sustain a temperature- and mercury-insensitive swelling in vitro. *Glia* 2000; 31:29–38.
64. Han Z, Wax MB, Patil RJ. Regulation of aquaporin-4 water channels by phorbol ester-dependent protein phosphorylation. *J Biol Chem* 1998; 273:6001–6004.
65. Nakahama K-I, Nagano M, Fujioka A, et al. Effect of TPA on Aquaporin-4 mRNA expression in cultured rat astrocytes. *Glia* 1999; 25:240–246.
66. Umenishi F, Verkman AS. Isolation of the human aquaporin-1 promoter and functional characterization in a human erythroleukemia cell line. *Genomics* 1998; 47:341–349.
67. Manley GT, Fujimura M, Ma T, et al. Aquaporin-4 deletion in mice reduces brain edema following acute water intoxication and ischemic stroke. *Nat Med* 2000; 6:159–163.
68. Ma T, Yang B, Gillespie A, et al. Generation and phenotype of a transgenic knock-out mouse lacking the mercurial-insensitive water channel aquaporin-4. *J Clin Invest* 1997; 100:957–962.
69. Solenov EI, Vetrivel L, Oshio K, et al. Optical measurement of swelling and water transport in spinal cord slices from aquaporin null mice. *J Neurosci Methods* 2002; 113(1):85–90.
70. Verkman AS. Potential utility of aquaporin blockers as aquaretics. *Drug News Perspect* 2001; 14:412–420.