FROM STRUCTURE TO DISEASE: THE EVOLVING TALE OF AQUAPORIN BIOLOGY

Landon S. King, David Kozono and Peter Agre

Abstract | Our understanding of the movement of water through cell membranes has been greatly advanced by the discovery of a family of water-specific, membrane-channel proteins — the aquaporins. These proteins are present in organisms at all levels of life, and their unique permeability characteristics and distribution in numerous tissues indicate diverse roles in the regulation of water homeostasis. The recognition of aquaporins has stimulated a reconsideration of membrane water permeability by investigators across a wide range of disciplines.

The existence of proteins that form water-specific membrane channels was postulated for several decades, largely on the basis of biophysical measurements of membrane permeability in red blood cells and in epithelial cells of the renal proximal tubule[1]. However, the identity of such channels remained unknown until approximately 12 years ago, when the serendipitous discovery of a red-blood-cell protein led to the description of aquaporin-1 (AQP1) as the first molecular water channel[2]. Water can be transported to varying degrees by other membrane proteins, for example, the Na^+/glucose co-transporter[3]. However, recognition of the unique properties of the aquaporins led to a paradigm shift in our consideration of membrane permeability: it is now known that water transport across the membrane can be regulated independently of solute transport. Recent reports from laboratories around the world have advanced our understanding of aquaporin biology, from the structural determinants of channel permeability to the assignment of their physiological function in different organs. These advances are the focus of this review.

The aquaporin protein family

Eleven mammalian aquaporins have been reported so far[4,5] (TABLE 1). Each has a unique cellular and subcellular distribution, with little overlap between homologues. The aquaporin family can be divided into two groups (FIG. 1) on the basis of their permeability characteristics, which generally coincide with specific amino-acid-sequence patterns. Most members of the first group (aquaporins) are only permeated by water, and this group includes AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8. AQP6 and AQP8 are in this group on the basis of sequence analysis, although AQP6 is permeated by anions[6] and AQP8 might be permeated by water and urea[7,8]. Members of the second group (aquaglyceroporins), which includes AQP3, AQP7, AQP9 and AQP10, are permeated by water to varying degrees, but are also permeated by other small solutes, in particular, glycerol. The bacterium *Escherichia coli* provides a model for this categorization, as it contains two aquaporin homologues — AqpZ, which is a water-permeable channel, and GlpF, which is a glycerol transporter (FIG. 1).

Why do we need so many aquaporins? The answer probably derives from the diverse requirements in different cells and organs for the regulation of water homeostasis. Aquaporins in the collecting duct of the kidney, capillaries in the lung and secretory cells in salivary glands (see below) are all capable of high rates of water transport. However, across the aquaporin family, differences have been identified in the transcriptional regulation of the genes, as well as in the post-translational modification, stability and polarized distribution of the proteins. As noted above, it is also evident that the

Department of Medicine, Division of Pulmonary and Critical Care Medicine, and the Department of Biological Chemistry, The Johns Hopkins University School of Medicine, 5501 Hopkins Bayview Circle, Baltimore, Maryland 21224, USA.
Correspondence to L.S.K.
e-mail: lsking@jhmi.edu
doi:10.1038/nrm1469

Table 1 | Permeability characteristics and predominant distribution for the known mammalian aquaporin homologues

Aquaporin	Permeability	Tissue distribution	Subcellular distribution*
AQP0	Water (low)	Lens	Plasma membrane
AQP1	Water (high)	Red blood cell, kidney, lung, vascular endothelium, brain, eye	Plasma membrane
AQP2	Water (high)	Kidney, vas deferens	Apical plasma membrane, intracellular vesicles
AQP3	Water (high), glycerol (high), urea (moderate)	Kidney, skin, lung, eye, colon	Basolateral plasma membrane
AQP4	Water (high)	Brain, muscle, kidney, lung, stomach, small intestine	Basolateral plasma membrane
AQP5	Water (high)	Salivary gland, lacrimal gland, sweat gland, lung, cornea	Apical plasma membrane
AQP6	Water (low), anions (NO_3^- > Cl^-)	Kidney	Intracellular vesicles
AQP7	Water (high), glycerol (high), urea (high), arsenite	Adipose tissue, kidney, testis	Plasma membrane
AQP8‡	Water (high)	Testis, kidney, liver, pancreas, small intestine, colon	Plasma membrane, intracellular vesicles
AQP9	Water (low), glycerol (high), urea (high), arsenite	Liver, leukocytes, brain, testis	Plasma membrane
AQP10	Water (low), glycerol (high), urea (high)	Small intestine	Intracellular vesicles

*Homologues that are present primarily in either the apical or basolateral membrane are noted as residing in one of these membranes, whereas homologues that are present in both of these membranes are described as having a plasma-membrane distribution. ‡AQP8 might be permeated by water and urea. AQP, aquaporin.

permeability characteristics differ among some members of the family. The complexity in the regulation of expression, membrane targeting and permeability necessitates the existence of more than just a single water-channel gene. Certainly, we must still expect to find new and surprising physiological functions for the aquaporins that require complex patterns of expression and regulation.

The unique aquaporin structure

Biochemical analyses of AQP1 revealed that the 28-kDa polypeptide that was evident on immunoblots represented the monomeric form of the protein, but that AQP1 (as well as other aquaporins) is present as a tetramer in the cell membrane[9]. FREEZE–FRACTURE studies also highlighted a tetrameric arrangement of the protein[10]. Recent studies have greatly enhanced our insights into the details of the aquaporin structure that direct this tetrameric arrangement, as well as into those features that dictate the permeability characteristics of the water channel.

Monomer structure. The signature sequence motif of the aquaporins is the three-amino-acid sequence NPA (Asn-Pro-Ala). One NPA motif is found in the amino-terminal half of each monomer, and a second NPA motif is found in the carboxy-terminal half[1] (FIG. 2a). When their amino termini are aligned, the overall percentage sequence identity among the aquaporin-family members is ~25–40%. However, it is much higher for the sequences that flank each of the NPA motifs. In addition to the similarity between the different aquaporins, the amino- and carboxy-terminal halves of AQP1 are related by their sequence, although these halves are oriented in opposite directions across the membrane bilayer (FIG. 2a). Hydropathy analysis of AQP1 indicated the presence of six transmembrane helices in each monomer[11]. In addition, mutational analysis of residues around the conserved NPA motifs led to predictions of an 'hourglass' structure, with two loops — the intracellular loop B and the extracellular loop E — folding into the membrane to form the pore[12] (FIG. 2a). Several recent structural studies — which include cryo-electron microscopy of human red-blood-cell AQP1 to 3.8-Å resolution[13,14] and X-ray crystal structures of GlpF[15] and bovine AQP1 (REF. 16) to 2.2-Å and 2.1-Å resolution, respectively — have confirmed the predicted hourglass structure. In addition, these studies have greatly advanced our understanding of the unique permeability characteristics of the aquaporins (see below).

Tetramer formation. Aquaporins are present in the membrane as tetramers, but, unlike ion channels, the channel for water permeability does not reside at the fourfold axis (the centre of the tetramer). Instead, each monomer contains a channel[12] (FIG. 2b). Structural studies have provided insights into the apparent requirement for tetramer formation[9]. The helices of each AQP1 monomer that are positioned on the outside face of the tetramer are hydrophobic, whereas those that are placed towards the centre of the tetramer are hydrophilic[17].

Channel selectivity and gating. Structural studies also revealed that the restriction of AQP1 permeability to water — excluding even hydronium (H_3O^+) ions — arises from two principal mechanisms[16–19]. First, the channels narrow to a diameter of 2.8 Å approximately 8 Å above the centre of the bilayer, which physically limits the size of molecules that can pass through them. A highly conserved arginine residue provides a fixed positive charge at this constriction site in each channel. The narrowest part of an *E. coli* GlpF channel is ~1 Å wider than in AQP1, and this increased diameter is sufficient to allow glycerol to pass through GlpF channels. Such

FREEZE–FRACTURE
A technique that allows the examination of membrane proteins by first freezing and then fracturing a tissue to separate the inner and outer leaflets of the membrane bilayer. This technique can be particularly useful for the examination of integral membrane proteins and cell junctions.

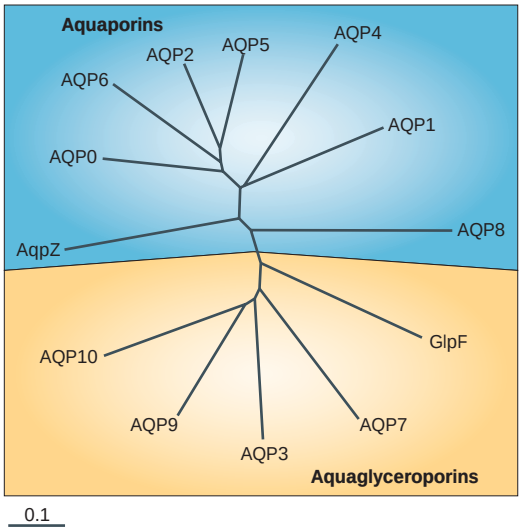

Figure 1 | **The aquaporin family tree.** This phylogenetic tree shows the relationship of the eleven human aquaporins (AQP0–AQP10), as well as the *Escherichia coli* aquaporin homologues AqpZ and GlpF. The assignments in the phylogenetic tree roughly correlate with permeability characteristics — the aquaporins are generally permeated only by water and the aquaglyceroporins are permeated by water and small solutes such as glycerol. The scale bar represents evolutionary distance: 0.1 equals 10 substitutions per 100 amino-acid residues (including reversions). So, the evolutionary distance, in terms of amino-acid substitutions, between two proteins is equal to the total distance along the path.

channel widening is predicted to occur in all aquaporin homologues that are permeated by small solutes like glycerol or urea. The second mechanism involves the orientation of a pair of DIPOLES at the NPA motifs. These dipoles interact with individual water molecules and prevent them from hydrogen bonding to adjacent water molecules. The functional separation of water molecules eliminates the possibility of H^+ transfer through a channel. The combination of size and charge restrictions provides the basis for the unique permeability characteristics of the aquaporins.

For several members of the aquaporin family, channel gating might have an important role in regulating permeability. The bovine form of AQP0, which was originally identified as major intrinsic protein (MIP) and is abundant in the lens of the eye, is activated at pH 6.0 when expressed in *Xenopus laevis* ooctyes[20]. A recent crystal structure of AQP0 highlighted two histidine residues within each pore that probably confer pH sensitivity[21]. In contrast to other aquaporins, AQP0 might also fulfil a structural role at the membrane junction between fibre cells in the lens. In double-layer AQP0 crystals, which are representative of the arrangement of AQP0 in the membrane junction, all of the subunits of a tetramer in one layer interact with two subunits of a tetramer in the adjacent layer. In this conformation, the pores were noted to be too narrow even for the passage of water, which indicates that membrane-junction formation stabilizes AQP0 in a closed conformation[21].

AQP3 permeability is reduced by low pH in oocytes[22], and by low pH and nickel in cultured lung epithelial cells[23]. A reduction in AQP4 water permeability following its activation by protein kinase C (PKC) has been reported in oocytes[24] and in renal epithelial cells[25]. However, AQP4 phosphorylation by PKC in proteoliposomes that had been reconstituted with purified AQP4 had no effect on water permeability (D.K. and P.A., unpublished observation). The water and ion permeability of AQP6 is activated by low pH and nitrate in oocytes[6] and cultured cells[26], and Yool and colleagues reported that the ion permeability of AQP1 that had been expressed in oocytes was gated by cyclic GMP[27]. However, the number of channels that are activated by cGMP could be as low as one AQP1 molecule per million[28], so the physiological implications of this result remain undefined. Together, though, these observations indicate that channel gating might allow the rapid alteration of membrane water permeability (and, in select cases, ion permeability) in specific tissues and conditions, and the structural determinants for gating these channels are the focus of intense interest.

Nakhoul *et al.*[29] and Cooper and Boron[30] reported that *X. laevis* oocytes that expressed human AQP1 showed an increase in membrane CO_2 permeability of approximately 40%. Prasad *et al.* reconstituted AQP1 into *E. coli* phospholipids and observed a fourfold increase in the rate of CO_2-induced intracellular acidification[31]. In all three studies, the increase in membrane CO_2 permeability was blocked by the addition of mercurial compounds, which is consistent with the inhibitory effects of mercury on AQP1-mediated water permeability. By contrast, Yang and colleagues examined the CO_2 permeability of red blood cells and the intact lungs of mice that had an *Aqp1* deletion, and they observed no differences between wild-type and *Aqp1*-null animals[32]. Fang *et al.* similarly observed no difference in the CO_2 permeability of intact lungs from wild-type, *Aqp1*-null and *Aqp5*-null mice[33]. Methodological differences in these studies remain unresolved, as do questions about the potential physiological implications of these observations for CO_2 transport in mammals[34,35]. Interestingly, this aspect of AQP-mediated permeability seems to be less ambiguous in plants. Uehlein and colleagues[36] recently reported that an aquaporin in tobacco plants increases CO_2 permeability and facilitates both photosynthesis and leaf growth, particularly when the CO_2 gradient is small.

Aquaporin physiology in health and disease

The genetic manipulation of rodents and the identification of humans with altered aquaporin genes have provided considerable insights into aquaporin-related physiology. The following sections describe the regulation and physiology of aquaporins in several organs in which they are believed to have fundamental roles.

Aquaporins in the kidney. The mammalian kidney (BOX 1) is the primary organ that regulates total body-water balance, and aquaporin-related physiology is most well

DIPOLE
A pair of equal and opposite electrical charges that are located only a short distance apart, for example, at either end of a small molecule.

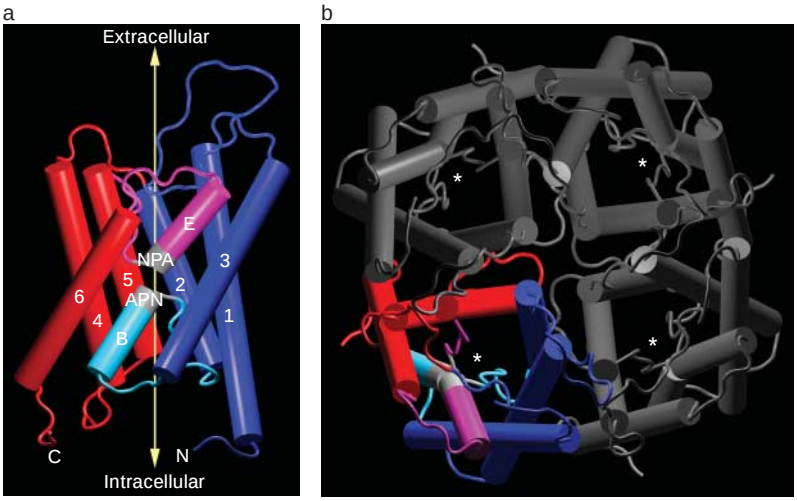

Figure 2 | **The structure of aquaporin-1. a** | The structure of the aquaporin-1 (AQP1) monomer is shown, with membrane-spanning helices numbered 1–6 and displayed as rods. The amino-terminal half of the molecule is shown in purple and light blue, and the carboxy-terminal half is shown in red and pink. Loops B and E, which fold into the membrane to form the pore, are labelled, as are the conserved NPA motifs (shown in light grey). Portions of loops B and E form α-helices, and are therefore shown as rods. The arrow highlights the route taken by water, which can move in both directions through the channel. **b** | The AQP1 tetramer, as seen from above. Asterisks denote the location of the water pore in each subunit. These structures are based on the Protein Data Bank coordinates 1J4N (REF. 11). C, carboxyl terminus; N, amino terminus.

understood in this organ. Each kidney contains approximately one million nephrons, and each nephron segment has a well-defined water permeability that correlates with the presence or absence of different aquaporins[37] (BOX 1; FIG. 3). In the proximal nephron, AQP1 is abundant in both the apical and basolateral membranes of the proximal tubule and the descending thin-limb epithelium, as well as in the endothelium of the descending vasa recta. AQP7 and AQP8 are also present in the proximal-tubule epithelium[37].

Recent investigations in both mice and humans confirmed a functional role for AQP1 in the kidney. Mice with a targeted knockout of *Aqp1* had increased urine output (polyuria) and a decreased urine-concentrating ability, as well as a decreased water permeability of the proximal tubule and the descending vasa recta. These functional consequences are consistent with predictions that were made on the basis of the distribution of this protein[38–40]. When deprived of water for 36 hours, *Aqp1*-null mice became profoundly dehydrated as a result of the urine-concentrating defect, and the SERUM OSMOLALITY increased from the normal value of approximately 310 mosmol kg^{-1} to nearly 500 mosmol kg^{-1}. The glomerular filtration rate was also reduced in these animals as a consequence of TUBULOGLOMERULAR FEEDBACK.

An extracellular epitope on AQP1 encodes the minor blood-group-antigen Colton[41], and seven Colton-null families have been identified worldwide. Individuals in three of the Colton-null families were found to be homozygous for distinct mutations in the *AQP1* gene and had a complete absence or marked deficiency of the AQP1 protein[42]. Surprisingly, these individuals suffered no obvious clinical consequences, besides their inability to tolerate blood transfusions from Colton-positive donors. So, what does this mean?

To examine the hypothesis that the absence of AQP1 would produce defects in water homeostasis under stress conditions, renal function was evaluated in two *AQP1*-null humans[43]. Both individuals had normal urine volumes, and normal indices of baseline renal function. When deprived of water, the *AQP1*-null individuals had normal increases in serum osmolality and vasopressin levels. However, both had a limited ability to concentrate urine, with the maximal urine osmolality being less than half as concentrated as in normal individuals following overnight water deprivation[44]. Although invasive micropuncture testing could not be carried out in these humans to confirm the segmental water permeability, measurements of proximal-tubule fluid reabsorption and the glomerular filtration rate were normal. This indicates that, in contrast to the *Aqp1*-null mice, the primary defect in these rare *AQP1*-null humans is not in the proximal tubule, but rather is in the descending thin limb and/or the descending vasa recta. Intrinsic differences between mice and humans with regard to maximal urine-concentrating ability are well established — mice can concentrate urine to greater than 3,000 mosmol kg^{-1}, whereas humans can maximally concentrate urine to ~1,200 mosmol kg^{-1}. Nonetheless, the manifestations of an AQP1 deficiency in mice are more severe than in *AQP1*-null humans, which indicates significant species-specific differences in the mechanisms of proximal-tubule water reabsorption. Alternatively, the extremely low frequency of this AQP1 deficiency in humans might indicate that these individuals have some, still unidentified, form of compensation.

Several water-channel proteins are expressed in the renal collecting duct (FIG. 3). For example, AQP2 is present in the water-absorbing principal cells of the renal collecting duct[37,45]. Vasopressin is the anti-diuretic hormone that is released from the pituitary gland and that stimulates urine concentration by increasing the water permeability of the collecting duct. After vasopressin binds to its receptor on collecting-duct epithelial cells, intracellular vesicles that contain AQP2 translocate to the apical membrane (FIG. 3), which markedly increases collecting-duct water permeability. These findings confirmed the classic 'shuttle-hypothesis' description of collecting-duct water permeability[46]. AQP2 is predominantly in the apical membrane of collecting-duct principal cells, but in the renal medulla — the most hypertonic part of the kidney — it can also be found on the basolateral membrane. van Balkom and colleagues showed that the exposure of a renal epithelial (Madin–Darby canine kidney, MDCK) cell line to a hypertonic medium for several days resulted in the insertion of AQP2 into the basolateral membrane[47]. Furthermore, the basolateral-membrane insertion of Aqp2 was observed in rats following chronic vasopressin exposure, which indicates that intrinsic targeting signals in AQP2 are not interpreted in the same way in all cells or under all conditions.

SERUM OSMOLALITY
A measure of the solute concentration in a particular solution, including biological solutions such as blood or urine. The units for osmolality are milliosmoles of solute per kg of solvent (mosmol kg^{-1}).

TUBULOGLOMERULAR FEEDBACK
The process by which increased fluid and solute delivery out of the proximal tubule feeds back to reduce glomerular filtration and to limit the loss of urine volume.

Box 1 | The mammalian kidney

The primary role of the kidney is to regulate the water balance of the body and the pH of the blood while eliminating toxic wastes. Each of the two kidneys contains approximately one million functional units, called nephrons. Each nephron is composed of several segments that have distinct roles in processing a filtrate of blood to generate urine. The water permeability of the different nephron segments is determined by the presence or absence of aquaporins at each site (FIG. 3). Blood is filtered at the glomerulus and this filtrate then passes into the lumen of the proximal tubule. This filtrate normally forms at a rate of 100–125 ml per min (the glomerular filtration rate), which results in a total of ~150–180 litres per day. Approximately 75% of this filtrate is resorbed by the epithelium of the proximal tubule and by the descending thin-limb segments, which contain aquaporins as well as transporters for various ions, glucose and other small molecules. Active solute transport (especially for Na^+, K^+ and Cl^-) takes place in the ascending limb of the loop of Henle. However, this part of the nephron is impermeable to water, and does not contain aquaporins. The final volume and concentration of urine is determined by the collecting duct, which is permeable to water only in the presence of the hormone vasopressin. This hormone binds to its receptor on principal cells of the collecting duct, and thereby regulates the plasma-membrane distribution of AQP2 for these cells (FIG. 3). Blood flows from the outer part of the kidney (cortex) into the inner part (medulla) by way of the descending vasa recta, and then back out of the kidney again by way of the ascending vasa recta. The vasa recta helps to maintain the high solute concentration of the interstitium of the kidney by transporting water that is resorbed by the proximal tubule and collecting duct out of the kidney.

Some of the fundamental components of the AQP2-trafficking mechanism have been established. Following the binding of vasopressin to its receptor, cyclic AMP levels increase[48] and AQP2 is phosphorylated on Ser256 by protein kinase A (REFS 49,50). AQP2-containing vesicles then undergo microtubule-mediated translocation to the apical membrane[51], where specific vesicle-docking proteins participate in membrane fusion[52–54]. The trafficking of AQP2 to intracellular vesicles following its synthesis does not require phosphorylation[55]. The apical-membrane targeting of AQP2 also requires the action of a heterotrimeric G-protein that is present in AQP2-containing vesicles; inhibiting the $G_{\alpha i3}$-subunit using inhibitory peptides or pertussis toxin blocks the apical-membrane insertion of AQP2 (REF. 56). Further complexities in the regulation of AQP2 membrane targeting have emerged. Klussman and colleagues showed that inhibiting Rho kinase led to AQP2 being targeted to the apical membrane even in the absence of vasopressin, which indicates that a combination of positive and negative signals might dictate vesicle trafficking[57]. The cGMP-mediated targeting of AQP2 to the plasma membrane has also been described[58]. The addition of cGMP analogues, nitric-oxide donors or atrial natriuretic factor to kidney slices or cultured renal epithelial cells increased the intracellular levels of cGMP and stimulated the apical-membrane insertion of AQP2 in the absence of vasopressin. As was observed for the cAMP-stimulated trafficking of AQP2, phosphorylation of Ser256 was required for this cGMP-stimulated apical-membrane targeting. It is not yet known whether protein kinase A or protein kinase G mediates AQP2 phosphorylation downstream of the cGMP signal.

The first example of a clinically important water-channel defect that altered the water permeability of an organ was provided by Deen and colleagues. They identified *AQP2* mutations in individuals with hereditary nephrogenic diabetes insipidus (NDI) — a rare disorder that results in the excretion of large volumes of dilute urine[59]. Mutations that produce both autosomal-dominant and autosomal-recessive inheritance patterns have been identified. In general, the autosomal-dominant mutations in *AQP2* produce trafficking defects[60] — heterotetramers, which are composed of mutant and normal AQP2 monomers, are not transported to the plasma membrane[61]. By contrast, the autosomal-recessive mutations lead to the misfolding of the mutant monomers, which are presumably degraded and therefore cannot oligomerize with the normal monomers. As a result, normal function is retained in heterozygotes.

Acquired NDI is common and has been observed in various clinical settings. Decreased AQP2 synthesis seems to be the final common result in several established animal models of acquired NDI, which include exposing rats to lithium[62], transient urinary obstruction[63] or chronic hypokalaemia[64]. At the other end of the water-imbalance spectrum, excessively high levels of AQP2 have been described in conditions of fluid retention, which include congestive heart failure, cirrhosis and pregnancy[65,66]. These observations confirm that AQP2 has a fundamental role in various pathological disorders of water homeostasis.

AQP3 and AQP4 are expressed in the basolateral membrane of collecting-duct principal cells (FIG. 3) — AQP3 is diffusely positioned along the collecting duct, whereas AQP4 is primarily localized to the inner-medullary collecting duct. *Aqp4*-deficient mice have a mild urine-concentrating defect[67]. *Aqp3*-null mice have severe polyuria, although the interpretation of this phenotype was complicated by a surprising, and unexplained, decrease in *Aqp2* expression in these animals[68]. Humans with *AQP4* mutations have not been identified so far. Although extremely rare, humans with *AQP3* mutations have recently been identified[69].

AQP6 is an intracellular water channel that resides in the intracellular vesicles of acid-secreting intercalated cells of the collecting duct[6] (FIG. 3). In these vesicles, AQP6 colocalizes with the H^+-ATPase, a protein that participates in the secretion of acid into the urine. In contrast to other aquaporins, AQP6 is permeated by anions as well as by water, and channel function is activated by low pH[6] and nitrate[26]. AQP6 probably participates in acid–base homeostasis by regulating the content of intercalated-cell vesicles.

Aquaporins in the lung. Water homeostasis is a crucial element of numerous pathophysiological processes that occur in the respiratory tract (BOX 2), and a network of aquaporins is present in this tract[70–73] (FIG. 4). In the upper respiratory tract, the epithelium of the nasopharynx and trachea show similar basic patterns of aquaporin expression in humans, rats and mice (FIG. 4). AQP3 is found in basal cells, AQP4 in the basolateral membrane of ciliated columnar cells, and AQP5 in the apical membrane of secretory cells in glands that are

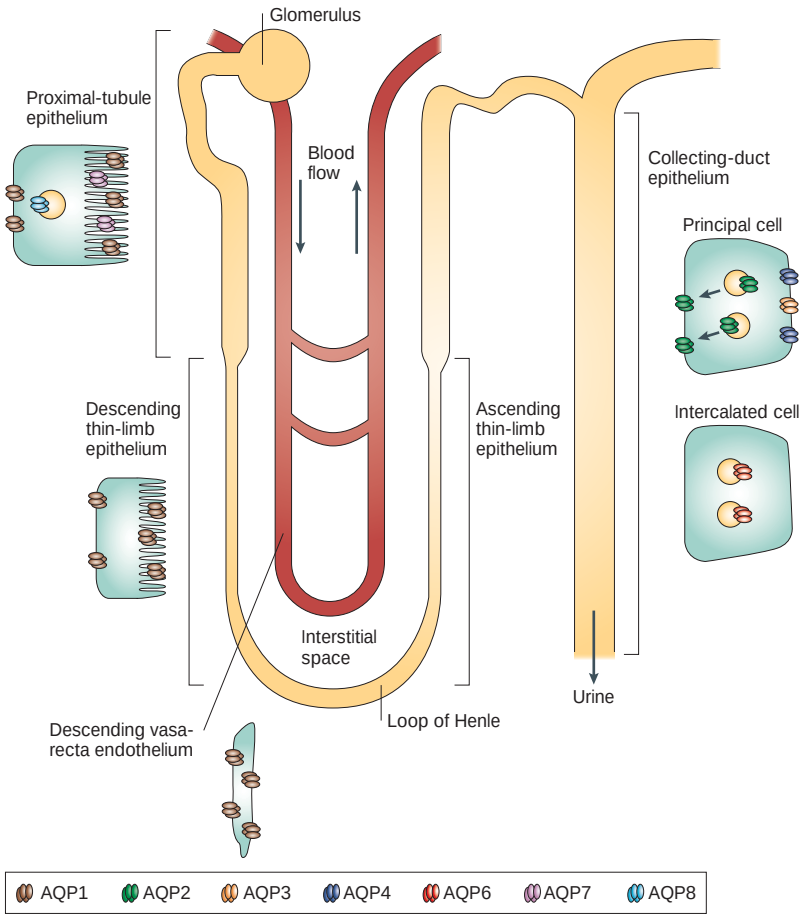

Figure 3 | **Aquaporin distribution in the human kidney.** A schematic of a nephron showing the aquaporin distribution in each of the nephron segments. Blood is filtered at the glomerulus, and the filtrate is modified as it travels through the nephron to make the final urine. Most of the glomerular filtrate is resorbed through aquaporin-1 (AQP1) in the proximal tubule and descending thin-limb epithelial cells, although AQP7 and AQP8 are also present in the proximal-tubule epithelium. Endothelial cells of the descending vasa recta contain AQP1, which facilitates the removal of water to maintain hypertonicity in the interstitial space. In the collecting duct, the hormone vasopressin increases membrane water permeability by stimulating the redistribution of AQP2 from cytoplasmic vesicles to the apical membrane of principal cells. AQP3 and AQP4 are present on the basolateral membrane of principal cells. AQP6 is present in intracellular vesicles of the acid-secreting intercalated cells. This figure is modified with permission from REF. 142 © (2002) Elsevier.

beneath the airway epithelium (submucosal glands). In human and mouse lungs, AQP5 is also present in the apical membrane of the surface epithelium (the ciliated columnar cells). AQP1 is abundant in endothelial cells of the capillaries and venules that surround the airways and the alveoli. In the alveolus, APQ5 is present in the apical membrane of type-I PNEUMOCYTES[70,74] and type-II pneumocytes might express AQP3.

Aquaporins in the respiratory tract are regulated at several levels. In rats, Aqp1 can be found in the lungs shortly before birth and its levels increase markedly during the perinatal period. Aqp3, Aqp4 and Aqp5, on the other hand, are only detectable after birth[75,76]. Corticosteroids induce *Aqp1* expression in both the fetal and adult lungs[75], at least in part, through transcriptional activation of the *Aqp1* promoter[77]. Ubiquitylation-mediated changes in the stability of AQP1 also contribute to changes in protein abundance[78]. In cultured lung epithelial cells, tumor necrosis factor-α reduced *AQP5* expression[79], whereas both cAMP[80] and hypertonicity[81] increased AQP5 abundance. Finally, the pH-dependent gating of AQP3 (REF. 23) is of great interest given that the normally slightly acidic surface layer[82] of the respiratory tract becomes markedly more acidic in pathological conditions such as asthma[83].

Several lines of evidence indicate functional roles for aquaporins in the airways of the lung. Submucosal glands in the airways contribute significantly to the generation of a liquid film that lines the airways[84]. This film —an aqueous phase that underlies a layer of mucous— helps to trap inhaled particulate matter and microorganisms, so that they can be swept out of the lung by cilia on the surface of cells. AQP5 is abundant in the apical membrane of secretory cells in these glands[70,71] and, compared to wild-type mice, *Aqp5*-null mice have reduced secretion from their airway submucosal glands[85]. Furthermore, Krane and colleagues observed that, compared to wild-type mice, *Aqp5*-null mice show a greater constriction of the airways in response to pharmacological stimulation with methacholine or acetylcholine[72] — that is, they are hyperresponsive to such agents. The mechanisms that underlie this observation are undefined, but both mechanical[86] and biochemical[87] links between changes in lumenal or interstitial water levels and resistance to airflow have been proposed. This is consistent with potential roles for water channels in the regulation of airway tone, and the clinical implications might be substantial given the worldwide prevalence of asthma.

Compared to wild-type animals, *Aqp1*-null mice have a tenfold decrease in the OSMOTIC WATER PERMEABILITY and a twofold decrease in the HYDROSTATIC PERMEABILITY across the lung vasculature[88], which is consistent with *Aqp1* being expressed in capillary endothelial cells[75,89]. Vascular permeability has recently been examined in *AQP1*-null humans, by adapting a technique that was used by Brown and colleagues to measure OEDEMA formation around the small airways[90]. In control individuals, intravenous infusion of a physiological saline solution increased the thickness of airway walls by 40–50%, which reflects early oedema formation around the bronchioles. In marked contrast, the thickness of airway walls did not change in *AQP1*-null individuals after fluid loading, which indicates that AQP1 contributes to the water permeability of the vascular membrane[89].

Isolated type-I pneumocytes that are expressing *Aqp5* have an extremely high water permeability[91], and *Aqp1*-null mice and *Aqp5*-null mice show a 10–30-fold decrease in the osmotic water permeability across the lung endothelium[92] and the alveolar epithelium[93], respectively. AQP1 and AQP5 levels are reduced in mouse lungs following adenoviral infection at a time when lung oedema increases[94] and *Aqp1* was identified as one of a limited number of candidate genes in nickel-induced lung injury[95]. These observations are consistent with aquaporins participating in the altered water homeostasis that is associated with lung injury.

PNEUMOCYTES
In common usage, this term is applied to the two principal epithelial cell types that line the alveoli of the lungs. Type-I pneumocytes are large flat cells, and type-II pneumocytes are cuboidal cells.

OSMOTIC WATER PERMEABILITY
The permeability of a membrane to water in response to an osmotic gradient across the membrane.

HYDROSTATIC PERMEABILITY
The permeability of a membrane in response to a pressure gradient across the membrane.

Box 2 | The mammalian respiratory tract

The lungs have the primary purpose of bringing oxygen from the inspired air into the bloodstream and eliminating carbon dioxide that is generated during metabolism from the bloodstream. The lungs are formed by a series of branching tubes that include the trachea, bronchi, bronchioles and alveolar sacs (FIG. 4). The trachea and bronchi in humans are defined by the presence of cartilage that supports the airway wall; bronchioles lack cartilage. The alveoli are the air sacs from which oxygen is taken up from the inspired air, and into which carbon dioxide is released to be exhaled. Gas exchange takes place principally in the alveoli. Epithelial cells that line the airways carry out various physiological functions, which include secreting water and mucus to form an airway surface layer and hydrating the inspired airstream. The airway epithelium is formed of several cell types, including: columnar cells that are ciliated; goblet cells that secrete mucus; and basal cells. The function of basal cells is incompletely defined, but they might function as progenitor cells for the rest of the epithelium. The alveolar sacs are formed by sheets of epithelial cells, which cover the capillaries that course through the alveolar walls. The alveolar epithelium has two principal cell types: large, flat, type-I pneumocytes that cover most of the surface of the alveolus; and cuboidal type-II pneumocytes that are metabolically active and secrete specialized lipids and proteins, which function as surfactants, into the alveoli. In addition to the capillaries that run through the alveolar walls and participate in gas exchange, the airways of the lung are surrounded by a dense vascular plexus that provides nutrients and participates in the fluid homeostasis of the interstitium around the airways.

OEDEMA
Excess fluid in a particular tissue or anatomic compartment. Swelling in the legs and excess fluid in the airspaces of the lung are examples of oedema.

CATARACTS
Opacities in the lens of the eye that are formed by precipitated proteins or degraded cells and that markedly decrease vision by interfering with the passage of light through the lens.

SCLERAL FIBROBLASTS
Fibroblasts that are found in the sclera, the tissue that forms the white part of the eye.

KERATINOCYTES
Epithelial cells of the skin that have differentiated to produce keratin. Keratinocytes are the predominant cell type in the epidermis of the skin.

TRABECULAR MESHWORK
An anatomic structure at the outer border of the anterior chamber of the eye. This structure consists of a network of endothelial-cell-covered strands that resorb the liquid that is found in the anterior chamber of the eye (aqueous humor).

CANALS OF SCHLEMM
Tubular channels in the eye that are found at the junction of the cornea and sclera, and through which aqueous humor drains.

Nonetheless, functional roles for aquaporins in oedema formation or resolution in alveoli have not yet been directly established. *Aqp5*-null mice resorb fluid out of their alveoli in a manner similar to wild-type mice[96]. Furthermore, pulmonary-oedema formation occurs similarly in wild-type mice and *Aqp1*- or *Aqp5*-null mice in response to several inflammatory stimuli[97]. Together these findings indicate that alveolar aquaporins might function in a limited context, for example, in cell-volume regulation, rather than in mediating transcellular fluid fluxes.

Aquaporins in the eye. Water homeostasis is crucial for the normal functioning of the eye — for example, for protecting the epithelium, for regulating intra-ocular fluid levels and pressure, and for maintaining the transparency of the pathway for light. A network of aquaporins in the eye carry out these functions[98] (FIG. 5).

AQP0 constitutes 50% of the total membrane protein in the fibre cells of the lens. Here, it is believed to have a structural role as a cell–cell adhesion molecule, in addition to functioning as a low-capacity water channel[99]. The gene that encodes AQP0 has been identified as the site of two naturally occurring mutations in mice that produce congenital bilateral CATARACTS[100].

Two families with dominantly-inherited cataracts have been found to contain different missense mutations in *AQP0* (REF. 101). These mutations — Glu134Gly or Thr138Arg — produced surprisingly distinct phenotypes in heterozygotes, despite their close proximity in the protein. Heterozygote individuals with the Glu134Gly substitution develop a single cataract in the lens of each eye that is stable after birth, whereas individuals with the Thr138Arg substitution have several small opacities in the lens that increase in number throughout life. The Glu134 and Thr138 residues are highly conserved among aquaporins. On the basis of the three-dimensional structure of AQP0 (REF. 21), both residues are located in the fourth transmembrane helix on the structurally important face inside each aqueous pore. When expressed in *X. laevis* oocytes, both substitutions impair AQP0 transport to the plasma membrane[102]. The development of cataracts in heterozygotes for each mutation indicates that AQP0 might have a structural role (for example, it might contribute to cell–cell adhesion): in the heterozygote state, a deficiency of essential cellular building blocks is more likely to become clinically apparent than a deficiency in the water-transporting activity of these proteins. A recent report of interactions between different AQP0 tetramers in AQP0 crystals is consistent with such a structural role[21]. It is anticipated that more subtle polymorphisms in *AQP0* will be found in some patients that have typical age-onset cataracts.

Aquaporins at other sites in the eye might also participate in the regulation of water homeostasis. AQP1 is present in SCLERAL FIBROBLASTS, the endothelium and KERATINOCYTES of the cornea, and the epithelium of the lens[98]. Consistent with a functional role in the corneal endothelium, *Aqp1*-null mice had reduced corneal thickness and a delayed recovery of transparency following the induction of corneal swelling by exposing the cornea to a hypotonic solution[103]. AQP1 and AQP4 are present in the non-pigmented epithelium of the anterior ciliary body, a structure that contributes to movement of fluid (aqueous humour) into the anterior chamber of the eye. In addition, AQP1 is present in the TRABECULAR MESHWORK and CANALS OF SCHLEMM, structures that resorb aqueous humour out of the anterior chamber[98]. Both *Aqp1*- and *Aqp4*-null mice have reduced intra-ocular pressure and fluid production, which is consistent with roles for aquaporins in anterior-chamber fluid dynamics[104].

AQP5 is expressed in the tear-secreting cells of the lacrimal gland, as well as the apical membrane of the corneal epithelium, in which it probably contributes to the generation of the surface liquid that helps to protect the cornea from mechanical injury[98,105]. The corneas of *Aqp5*-null mice are markedly thicker than the corneas of wild-type or *Aqp1*-null mice and, compared to wild-type animals, corneal epithelial cells from *Aqp5*-null mice have reduced rates of cell-volume change in response to osmotic gradients[103]. Other water channels are also expressed in the eye. AQP3 is found in the epithelium that covers the outer margins of the eye (the bulbar conjunctival epithelium or conjunctiva) and might help to lubricate the surface of the eye. AQP4 is present in two cell types in the retina — Müller cells and glial cells. Here, it probably contributes to the regulation of extracellular osmolality that fluctuates with neuronal activity[98].

Aquaporins in secretory glands. Secretion from salivary glands, lacrimal (tear) glands and sweat glands is differentially regulated. However, in each gland, secretions are generated by coupling active electrolyte transport to water flow, and AQP5 can be found at the apical membrane of secretory cells in each of these glands[70,106–109]. Ma and colleagues showed that mice with an *Aqp5*

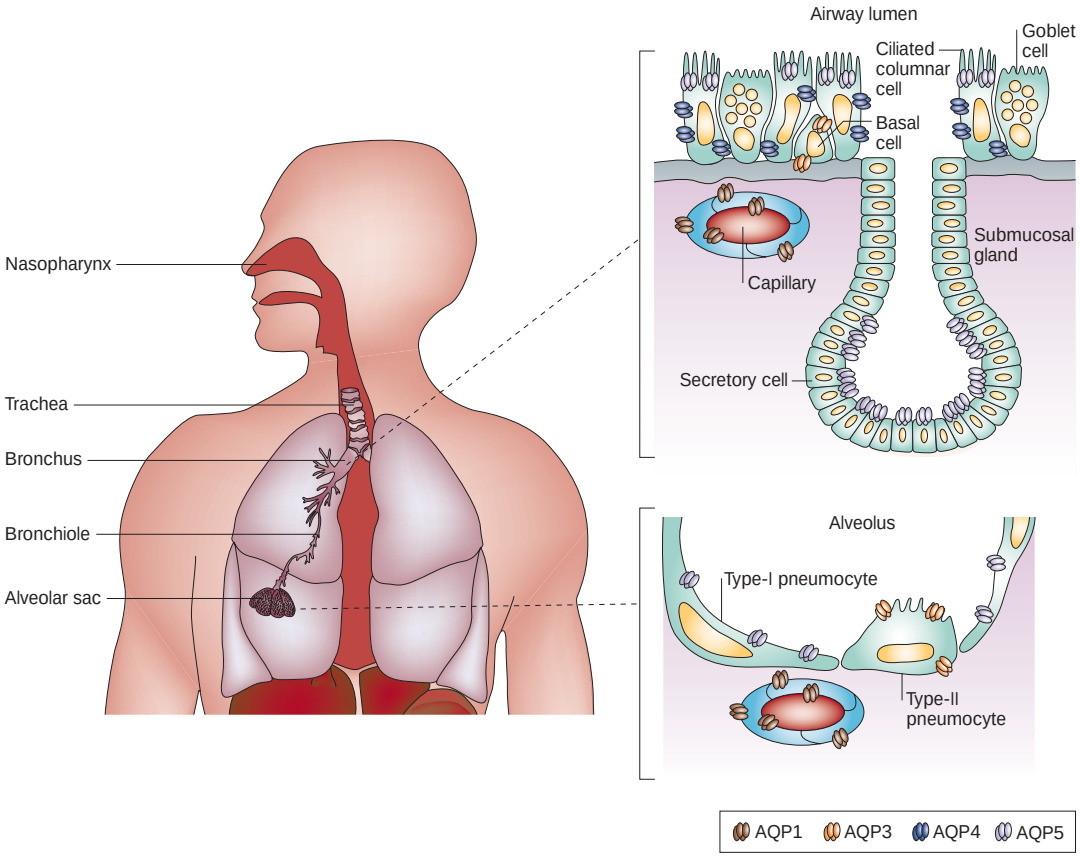

Figure 4 | **Aquaporin distribution in the human respiratory tract.** The epithelium that lines the upper airways contains three aquaporins. Aquaporin-5 (AQP5) is in the apical membrane of the airway epithelial cells (the ciliated columnar cells), AQP4 is in the basolateral membrane of these cells, and AQP3 is present in the basal cells of the epithelium. AQP1 is found in the apical and basolateral membranes of the endothelial cells of the capillaries and venules that surround the airways. Submucosal glands open onto the airway surface and the secretory cells of these glands have AQP5 in their apical membrane. In the alveoli, or air sacs of the lung, AQP5 is in the apical membrane of type-I pneumocytes, and AQP1 can be found in capillary endothelial cells. Type-II pneumocytes might express AQP3.

deletion have decreased salivation following stimulation with pilocarpine and that the saliva from these mice was hypertonic (~450 mosmol kg^{-1})[110]. Krane and colleagues also observed a decreased salivation in *Aqp5*-null mice following stimulation using pilocarpine, although, in these studies, the saliva was hypotonic (~250 mosmol kg^{-1})[111]. The basis for this difference is not known — pilocarpine dosing was different in the two studies, but mouse-strain-related differences in the compensation and response mechanisms would seem a more probable explanation. The success of defining functional roles for AQP5 in other secretory glands using mouse-knockout models has been more variable. Nejsum and co-workers showed decreased secretion from forepaw sweat glands in *Aqp5*-null mice two minutes after the local administration of pilocarpine[112]. However, Song *et al.* observed no differences in sweat-gland secretion in *Aqp5*-null mice compared to wild-type mice five and ten minutes after the systemic administration of pilocarpine[113]. Lacrimal-gland secretion was also not diminished in this strain of *Aqp5*-null mice[114]. Differences in timing, pilocarpine administration and mouse strain might explain the apparent discrepancy in sweat-gland function.

Krane and colleagues also showed that isolated salivary gland (sublingual and parotid gland) secretory cells from *Aqp5*-null mice had a reduced membrane water permeability, as well as a reduced rate of return to their original cell volume after a period of swelling that was induced by hypotonic challenge (a process called regulatory-volume decrease)[111]. Similar observations were made *in vivo* in the corneas of *Aqp1*-null mice[103]. Recently, aquaporin levels have been shown to be dynamically regulated during osmotic stress, by mitogen-activated-protein-kinase-mediated signalling[81,115] and by changes in ubiquitylation and protein stability[78], which further highlights fundamental roles for aquaporins in the cellular response to osmotic stress.

Sjögren's syndrome is an autoimmune disease, the principal clinical manifestations of which are dry eyes and a dry mouth. Expression of AQP5 in salivary and lacrimal glands indicated a potential role for this aquaporin in the pathophysiology of Sjögren's syndrome. Steinfeld and colleagues examined the AQP5

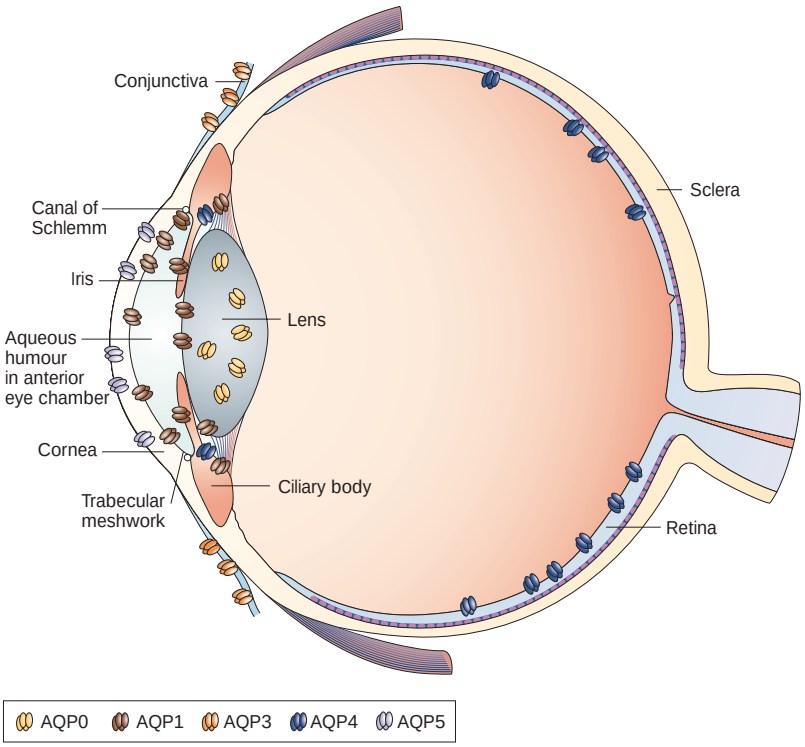

Figure 5 | **Aquaporin distribution in the human eye.** AQP0 is present in the fibre cells of the lens. AQP1 is present in scleral fibroblasts (not shown), in the epithelial cells that cover the lens, and in the endothelial cells that line the blood vessels at the back of the cornea. It is also present in the epithelium of the anterior ciliary body and in the trabecular meshwork and canals of Schlemm, which all contribute to aqueous-humour formation and resorption in the anterior chamber of the eye. The epithelium covering the outer margins of the eye, called the conjunctiva, contains AQP3. AQP4 is present in specialized cell types of the retina, called glial cells and Müller cells, as well as in the epithelium of the anterior ciliary body. AQP5 is present in the secretory cells of the tear-forming lacrimal glands (not shown), and also in the apical membrane of corneal epithelial cells.

distribution in minor salivary-gland biopsies of control individuals and those with Sjögren's syndrome, and found a decreased labelling for AQP5 in the apical membrane of salivary-gland secretory cells in the latter individuals[107]. Tsubota *et al*. compared lacrimal-gland biopsies of individuals with Sjögren's syndrome with those of three different control groups[108] and, consistent with the results of Steinfeld and colleagues, found that individuals with Sjögren's syndrome had a marked decrease in AQP5 levels at the apical membrane of lacrimal secretory cells. Tsubota *et al*. further showed that the distribution of the Na^+/K^+-ATPase in the basolateral membrane and an Na^+ channel in the apical membrane were normal in Sjögren's syndrome patients, as was the level of AQP5 production. These studies indicate that some individuals with Sjögren's syndrome have an AQP5 transport defect that probably contributes to the decrease in salivation and lacrimation.

However, Sjögren's syndrome might prove to be a heterogeneous disease, because a third group of investigators carried out minor salivary-gland biopsies on a larger cohort of patients with Sjögren's syndrome and found no difference in the AQP5 distribution compared to control subjects[109]. Interestingly, salivary-gland biopsies from these patients were found to have reduced AQP1 levels in MYOEPITHELIAL CELLS. Despite this complexity, determination of the mechanisms that lead to the apical targeting of AQP5 in secretory glands might provide the basis for designing therapies to correct AQP5 transport and to restore gland secretion in a subset of patients with Sjögren's syndrome.

Aquaporins in the brain. The tight regulation of intracerebral water content is crucial for brain function, especially given the rigid physical constraint that is imposed by the bony cranium. The brain is composed of two main types of cell — neurons (nerve cells), which receive and transmit signals in the brain; and glial cells, which carry out various roles to support the neurons. Aquaporins are expressed in glial cells at sites that are indicative of a role for them in water homeostasis in the brain[116–118].

Astrocytes, a type of glial cell, form plasma-membrane projections that surround blood vessels in the brain. They are believed to contribute to the specialized properties of the blood–brain barrier that limit its permeability to water and macromolecules. The astrocyte projections around the blood vessels, called foot processes, express *AQP4* abundantly[117] (FIG. 6). Furthermore, Amiry-Moghaddam and colleagues recently showed that brain endothelial cells also express *AQP4* (REF. 119), which is the first description of any aquaporin in blood vessels in the brain. The polarized distribution of AQP4 to the astrocyte foot process at the blood–brain interface is facilitated by the interaction of AQP4 with a large complex of proteins that connect the cell cytoskeleton to the extracellular matrix[120]. Three amino acids at the carboxyl terminus of AQP4 form a specialized protein-binding domain called a PDZ domain (PSD95, Discs-large, Zona-occludens-1 domain)[121]. The PDZ domain of AQP4 binds to syntrophin, a component of the protein complex that tethers AQP4 to the correct location at the blood–brain barrier[120]. Studies of AQP4 targeting in epithelial cells indicate that further sequences in the carboxyl terminus, including a dileucine repeat and a tyrosine-based motif, might be required for basolateral targeting[122]. Syntrophin-null mice have normal levels of AQP4, but this AQP4 is distributed throughout the astrocyte, rather than being concentrated in the membrane that is adjacent to the blood–brain barrier. Another member of this protein complex — dystrophin — has been associated with the muscle disease muscular dystrophy that can produce profound weakness[123]. In a mouse model of muscular dystrophy, dystrophin-deficient mice have reduced AQP4 levels in the astrocyte foot processes and an increased swelling of perivascular astrocyte processes[124,125]. These observations indicate that disrupting the mechanisms that determine the polarized targeting of AQP4 in astrocytes might contribute to alterations in water homeostasis in the brain.

Roles for AQP4 in water homeostasis in the brain were first indicated by studies of *Aqp4*-null mice, which had reduced brain oedema compared to wild-type animals following acute water intoxication or ischaemic

MYOEPITHELIAL CELLS
Contractile epithelial-like cells that function like smooth muscle, but are usually found between secretory cells and the basement membrane in glands. They have long cytoplasmic extensions, which contain actin bands that can contract to facilitate fluid movement out of the secretory gland.

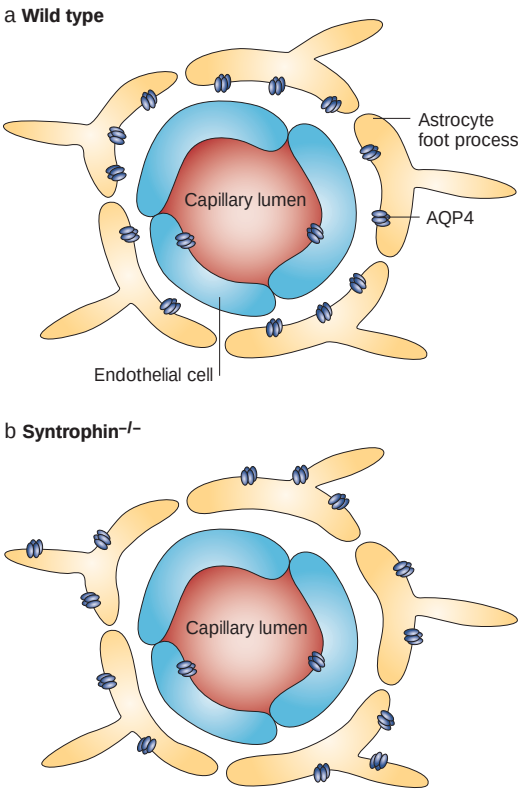

Figure 6 | **Aquaporin expression in brain astrocytes.** **a** | The foot processes of astrocytes surround the capillary endothelial cells. AQP4 is present in the astrocyte membrane that is adjacent to the endothelium and, at a lower level, in the endothelial cell membrane. **b** | In syntrophin-null mice, AQP4 targeting to the peri-endothelial astrocyte foot process is disrupted, and AQP4 is distributed to other sites in the astrocyte. The vascular expression of AQP4 is preserved.

stroke[126]. This observation is supported by studies of syntrophin-null mice and dystrophin-null mice, both of which show a mislocalization of AQP4 from astroglial foot processes, in conjunction with a partial resistance to oedema after brain injury[125,127]. Differences in the pathophysiological role of AQP4 in water homeostasis in the brain during acute versus chronic conditions will be of great interest given the often catastrophic consequences of brain oedema following acute brain injury. Further roles for water channels in the brain will probably emerge. AQP1 is expressed in the apical membrane of the CHOROID PLEXUS epithelium[128] and AQP9 is expressed in astrocytes[129]. The functional roles of these water channels in the brain have not yet been defined.

Expanding roles for aquaporins

Recent work by numerous investigators has extended the reach of aquaporin biology. AQP3 is expressed in skin keratinocytes, and *Aqp3*-null mice show markedly impaired hydration of the stratum corneum layer of the skin[130]. In *Aqp3*-null mice, both skin hydration and barrier function were improved following the administration of topical or oral glycerol, which indicates that AQP3-mediated glycerol transport is a crucial element of these basic skin functions[131]. Burghardt and colleagues have defined aquaporin distribution in the human pancreas[132], where AQP8 in secretory cells, and AQP1 and AQP5 in the proximal ducts, colocalized with the CYSTIC FIBROSIS TRANSMEMBRANE-CONDUCTANCE REGULATOR, which highlights potential roles for aquaporins in the regulation of pancreatic exocrine secretions. Several aquaporins have been identified in different structures of the ear[133,134], and mice with an *Aqp4* deletion have varying degrees of hearing deficits, which can range up to deafness depending on the mouse strain[135]. Richard and colleagues showed that *Aqp5* expression is induced in mouse intra-uterine glands during the early stages of pregnancy, and that *Aqp8* and *Aqp9* are expressed in non-overlapping distributions in the implanting blastocyst, which is consistent with roles for aquaporins in the earliest stages of pregnancy[136]. The ontogeny and distribution of AQP7 and AQP8 in developing spermatocytes indicate potential roles in male fertility[137].

Potential roles for aquaporins in carbohydrate metabolism are also emerging. AQP7 is present in adipocytes[138], and is permeated by glycerol. Kondo and colleagues showed that an individual with an *AQP7* mutation that blocked *in vitro* glycerol transport failed to produce an increase in blood glycerol levels following exercise — an apparent consequence of reduced glycerol efflux from adipocytes[139]. AQP9 is abundant in hepatocytes of the liver, and is also permeated by glycerol. Rats that were fasted or made diabetic by intravenous streptozotocin administration showed up to a 20-fold increase in AQP9 abundance in liver[140]. This increase was blocked by the administration of insulin and by the correction of blood glucose in the diabetic animals. Although still speculative, these findings indicate the potential importance of aquaporin-mediated glycerol transport out of adipocytes and into the liver to support GLUCONEOGENESIS in the fasted state. Interestingly, AQP7 and AQP9 are also permeated by arsenite and might contribute to the toxicity of arsenic ingestion[141].

As the story unfolds

The discovery of aquaporins has greatly enhanced our understanding of the molecular basis of the transport of water across membranes, and has prompted a reconsideration of the determinants of the water permeability of membranes. It is now evident that membrane water permeability can be regulated independently of solute permeability. Elucidation of the structural determinants of pore specificity should facilitate the design and discovery of channel-modulating agents. Studies of gene expression, as well as studies of protein stability, might reveal mechanisms for manipulating the level of aquaporin expression. The degree to which aquaporins determine membrane water permeability in specific tissues probably varies depending on both the organ and the context. However, as their physiological roles are defined, it will become increasingly appealing to consider aquaporins as specific therapeutic targets for various pathophysiological conditions in which the disruption of water homeostasis is a principal manifestation.

CHOROID PLEXUS
A collection of villous-like processes at select sites in the ventricular system of the brain. These processes contain a special secretory epithelium that secretes cerebrospinal fluid.

CYSTIC FIBROSIS TRANSMEMBRANE-CONDUCTANCE REGULATOR (CFTR). This protein forms a chloride channel, is present in many tissues including the lung, kidney and pancreas, and is mutated in cystic fibrosis.

GLUCONEOGENESIS
The process by which glucose is made from amino acids or glycerol in the fasting state. This process occurs primarily in the liver.

1. Agre, P. *et al.* Aquaporin CHIP: the archetypal molecular water channel. *Am. J. Physiol.* **265**, F463–F476 (1993).
2. Preston, G. M., Carroll, T. P., Guggino, W. B. & Agre, P. Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein. *Science* **256**, 385–387 (1992).
This is the initial report of the discovery and biophysical characterization of aquaporin-1, the first molecular water channel to be identified.
3. Loo, D. D., Wright, E. M. & Zeuthen, T. Water pumps. *J. Physiol.* **542**, 53–60 (2002).
4. Borgnia, M., Nielsen, S., Engel, A. & Agre, P. Cellular and molecular biology of the aquaporin water channels. *Annu. Rev. Biochem.* **68**, 425–458 (1999).
5. Koyama, Y. *et al.* Molecular cloning of a new aquaporin from rat pancreas and liver. *J. Biol. Chem.* **272**, 30329–30333 (1997).
6. Yasui, M. *et al.* Rapid gating and anion permeability of an intracellular aquaporin. *Nature* **402**, 184–187 (1999).
This report highlights the unusual features of AQP6, which, in contrast to most members of the aquaporin family, is an intracellular channel, is gated and is permeated by anions as well as by water.
7. Ishibashi, K. *et al.* Cloning and functional expression of a second new aquaporin abundantly expressed in testis. *Biochem. Biophys. Res. Commun.* **237**, 714–718 (1997).
8. Ma, T., Yang, B. & Verkman, A. S. Cloning of a novel water and urea-permeable aquaporin from mouse expressed strongly in colon, placenta, liver, and heart. *Biochem. Biophys. Res. Commun.* **240**, 324–328 (1997).
9. Smith, B. L. & Agre, P. Erythrocyte Mr 28,000 transmembrane protein exists as a multisubunit oligomer similar to channel proteins. *J. Biol. Chem.* **266**, 6407–6415 (1991).
10. Verbavatz, J. M. *et al.* Tetrameric assembly of CHIP28 water channels in liposomes and cell membranes: a freeze–fracture study. *J. Cell Biol.* **123**, 605–618 (1993).
11. Preston, G. M. & Agre, P. Isolation of the cDNA for erythrocyte integral membrane protein of 28 kilodaltons: member of an ancient channel family. *Proc. Natl Acad. Sci. USA* **88**, 11110–11114 (1991).
12. Jung, J. S., Preston, G. M., Smith, B. L., Guggino, W. B. & Agre, P. Molecular structure of the water channel through aquaporin CHIP. The hourglass model. *J. Biol. Chem.* **269**, 14648–14654 (1994).
13. Murata, K. *et al.* Structural determinants of water permeation through aquaporin-1. *Nature* **407**, 599–605 (2000).
14. Ren, G., Reddy, V. S., Cheng, A., Melnyk, P. & Mitra, A. K. Visualization of a water-selective pore by electron crystallography in vitreous ice. *Proc. Natl Acad. Sci. USA* **98**, 1398–1403 (2001).
15. Fu, D. *et al.* Structure of a glycerol-conducting channel and the basis for its selectivity. *Science* **290**, 481–486 (2000).
16. Sui, H., Han, B. G., Lee, J. K., Walian, P. & Jap, B. K. Structural basis of water-specific transport through the AQP1 water channel. *Nature* **414**, 872–878 (2001).
References 15 and 16 define the atomic structures of GlpF and AQP1, respectively, which are both members of the aquaporin family of proteins.
17. Kozono, D., Yasui, M., King, L. S. & Agre, P. Aquaporin water channels: atomic structure and molecular dynamics meet clinical medicine. *J. Clin. Invest.* **109**, 1395–1399 (2002).
18. de Groot, B. L. & Grubmuller, H. Water permeation across biological membranes: mechanism and dynamics of aquaporin-1 and GlpF. *Science* **294**, 2353–2357 (2001).
19. Tajkhorshid, E. *et al.* Control of the selectivity of the aquaporin water channel family by global orientational tuning. *Science* **296**, 525–530 (2002).
20. Nemeth-Cahalan, K. L. & Hall, J. E. pH and calcium regulate the water permeability of aquaporin 0. *J. Biol. Chem.* **275**, 6777–6782 (2000).
21. Gonen, T., Sliz, P., Kistler, J., Cheng, Y. & Walz, T. Aquaporin-0 membrane junctions reveal the structure of a closed water pore. *Nature* **429**, 193–197 (2004).
22. Zeuthen, T. & Klaerke, D. A. Transport of water and glycerol in aquaporin 3 is gated by H^+. *J. Biol. Chem.* **274**, 21631–21636 (1999).
23. Zelenina, M., Bondar, A. A., Zelenin, S. & Aperia, A. Nickel and extracellular acidification inhibit the water permeability of human aquaporin-3 in lung epithelial cells. *J. Biol. Chem.* **278**, 30037–30043 (2003).
24. Han, H., Wax, M. B. & Patil, R. V. Regulation of aquaporin-4 water channels by phorbol ester-dependent protein phosphorylation. *J. Biol. Chem.* **273**, 6001–6004 (1998).
25. Zelenia, M., Zelenin, S., Bondar, A. A., Brismar, H. & Aperia, A. Water permeability of aquaporin-4 is deceased by protein kinase C and dopamine. *Am. J. Physiol.* **283**, F309–F318 (2002).
26. Ikeda, M. *et al.* Characterization of aquaporin-6 as a nitrate channel in mammalian cells. *J. Biol. Chem.* **277**, 39873–39879 (2002).
27. Anthony, T. L. *et al.* Cloned human aquaporin-1 is a cyclic GMP-gated ion channel. *Mol. Pharm.* **57**, 576–588 (2000).
28. Saparov, S. M., Kozono, D., Rothe, U., Agre, P. & Pohl, P. Water and ion permeation of aquaporin-1 in planar lipid bilayers. *J. Biol. Chem.* **276**, 31515–31520 (2001).
29. Nakhoul, N. L., Davis, B. A., Romero, M. F. & Boron, W. F. Effect of expressing the water channel aquaporin-1 on the CO_2 permeability of *Xenopus* oocytes. *Am. J. Physiol.* **274**, C543–548 (1998).
30. Cooper, G. J. & Boron, W. F. Effect of PCMBS on CO_2 permeability of *Xenopus* oocytes expressing aquaporin-1 or its C189S mutant. *Am. J. Physiol.* **275**, C1481–C1486 (1998).
31. Prasad, G. V., Coury, L. A., Fin, F. & Zeidel, M. L. Reconstituted aquaporin 1 water channels transport CO_2 across membranes. *J. Biol. Chem.* **273**, 33123–33126 (1998).
32. Yang, B. *et al.* Carbon dioxide permeability of aquaporin-1 measured in erthrocytes and lung of aquaporin-1 null mice and in reconstituted liposomes. *J. Biol. Chem.* **275**, 2686–2692 (2000).
33. Fang, X., Yang, B., Matthay, M. A. & Verkman, A. S. Evidence against aquaporin-1 dependent CO_2 permeability in lung and kidney. *J. Physiol.* **542**, 63–69 (2002).
34. Cooper, G. J., Zhou, Y., Bouyer, P., Grichtchenko, I. I. & Boron, W. F. Transport of volatile solutes through AQP1. *J. Physiol.* **542**, 17–29 (2002).
35. Verkman, A. S. Does aquaporin-1 pass gas? An opposing view. *J. Physiol.* **542**, 31 (2002).
36. Uehlein, N., Lovisolo, C., Siefritz, F. & Kaldenhoff, R. The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. *Nature* **425**, 734–737 (2003).
Describes the physiological impact of aquaporin-mediated CO_2 permeability on photosynthesis and leaf growth in plants.
37. Nielsen, S. *et al.* Aquaporins in the kidney: from molecules to medicine. *Physiol. Rev.* **82**, 205–244 (2002).
38. Ma, T. *et al.* Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. *J. Biol. Chem.* **273**, 4296–4299 (1998).
This description of renal function in *Aqp1*-null mice provided convincing evidence for a physiological role for AQP1 in the kidney.
39. Schnermann, J. *et al.* Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc. Natl Acad. Sci. USA* **95**, 9660–9664 (1998).
40. Pallone, T. L., Edwards, A., Ma, T., Silldorff, E. P. & Verkman, A. S. Requirement of aquaporin-1 for NaCl-driven water transport across descending vasa recta. *J. Clin. Invest.* **105**, 215–222 (2000).
41. Smith, B. L., Preston, G. M., Spring, F. A., Anstee, D. J. & Agre, P. Human red cell aquaporin CHIP. I. Molecular characterization of ABH and Colton blood group antigens. *J. Clin. Invest.* **94**, 1043–1049 (1994).
42. Preston, G. M., Smith, B. L., Zeidel, M. L., Moulds, J. J. & Agre, P. Mutations in aquaporin-1 in phenotypically normal humans without functional CHIP water channels. *Science* **265**, 1585–1587 (1994).
43. King, L. S., Choi, M., Fernandez, P. C., Cartron, J. P. & Agre, P. Defective urinary-concentrating ability due to a complete deficiency of aquaporin-1. *N. Engl. J. Med.* **345**, 175–179 (2001).
44. Robertson, G. L. Differential diagnosis of polyuria. *Annu. Rev. Med.* **39**, 425–442 (1988).
45. Brown, D. The ins and outs of aquaporin-2 trafficking. *Am. J. Physiol.* **284**, F893–F901 (2003).
46. Wade, J. B., Stetson, D. L. & Lewis, S. A. ADH action: evidence for a membrane shuttle mechanism. *Ann. NY Acad. Sci.* **372**, 106–117 (1981).
47. van Balkom, B. W. *et al.* Hypertonicity is involved in redirecting the aquaporin-2 water channel into the basolateral, instead of the apical, plasma membrane of renal epithelial cells. *J. Biol. Chem.* **278**, 1101–1107 (2003).
48. Edwards, R. M., Jackson, B. A. & Dousa, T. P. ADH-sensitive cAMP system in papillary collecting duct: effect of osmolality and PGE2. *Am. J. Physiol.* **240**, F311–F318 (1981).
49. Fushimi, K., Sasaki, S. & Marumo, F. Phosphorylation of serine 256 is required for cAMP-dependent regulatory exocytosis of the aquaporin-2 water channel. *J. Biol. Chem.* **272**, 14800–14804 (1997).
50. Katsura, T., Gustafson, C. E., Ausiello, D. A. & Brown, D. Protein kinase A phosphorylation is involved in regulated exocytosis of aquaporin-2 in transfected LLC-PK1 cells. *Am. J. Physiol.* **272**, F817–F822 (1997).
51. Marples, D. *et al.* Dynein and dynactin colocalize with AQP2 water channels in intracellular vesicles from kidney collecting duct. *Am. J. Physiol* **274**, F384–F394 (1998).
52. Nielsen, S. *et al.* Expression of VAMP-2-like protein in kidney collecting duct intracellular vesicles. *J. Clin. Invest.* **96**, 1834–1844 (1995).
53. Inoue, T. *et al.* SNAP-23 in rat kidney: colocalization with aquaporin-2 in collecting duct vesicles. *Am. J. Physiol.* **275**, F752–F760 (1998).
54. Gouraud, S. *et al.* Functional involvement of VAMP/synaptobrevin-2 in cAMP-stimulated aquaporin 2 translocation in renal collecting duct cells. *J. Cell Sci.* **115**, 3667–3674 (2002).
55. van Balkom, V. W. M. *et al.* The role of putative phosphorylation sites in the targeting and shuttling of the aquaporin-2 water channel. *J. Biol. Chem.* **277**, 41473–41479 (2003).
56. Valenti, G. *et al.* A heterotrimeric G protein of the G_1 family is required for cAMP-triggered trafficking of aquaporin 2 in kidney epithelial cells. *J. Biol. Chem.* **273**, 22627–22634 (1998).
57. Klussmann, E. *et al.* An inhibitory role of rho in the vasopressin-mediated translocation of aquaporin-2 into cell membranes of renal principal cells. *J. Biol. Chem.* **276**, 20451–20457 (2001).
58. Bouley, R. *et al.* Nitric oxide and atrial natriuretic factor stimulate cGMP-dependent membrane insertion of aquaporin 2 in renal epithelial cells. *J. Clin. Invest.* **106**, 1115–1126 (2000).
59. Deen, P. M. *et al.* Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science* **264**, 92–95 (1994).
These investigators provided the first example of an aquaporin mutation that has physiological consequences in humans.
60. Mulders, S. M. *et al.* An aquaporin-2 water channel mutant which causes autosomal dominant nephrogenic diabetes insipidus is retained in the Golgi complex. *J. Clin. Invest.* **102**, 57–66 (1998).
61. Kamsteeg, E. J., Wormhoudt, T. A., Rijss, J. P., van Os, C. H. & Deen, P. M. An impaired routing of wild-type aquaporin-2 after tetramerization with an aquaporin-2 mutant explains dominant nephrogenic diabetes insipidus. *EMBO J.* **18**, 2394–2400 (1999).
62. Marples, D., Christensen, S., Christensen, E. I., Ottosen, P. D. & Nielsen, S. Lithium-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla. *J. Clin. Invest.* **95**, 1838–1845 (1995).
63. Frøkiær, J., Marples, D., Knepper, M. A. & Nielsen, S. Bilateral ureteral obstruction downregulates expression of vasopressin-sensitive AQP-2 water channel in rat kidney. *Am. J. Physiol.* **270**, F657–F668 (1996).
64. Marples, D., Frøkiær, J., Dorup, J., Knepper, M. A. & Nielsen, S. Hypokalemia-induced downregulation of aquaporin-2 water channel expression in rat kidney medulla and cortex. *J. Clin. Invest.* **97**, 1960–1968 (1996).
65. Nielsen, S. *et al.* Congestive heart failure in rats is associated with increased expression and targeting of aquaporin-2 water channel in collecting duct. *Proc. Natl Acad. Sci. USA* **94**, 5450–5455 (1997).
66. Schrier, R. W. & Martin, P. Y. Recent advances in the understanding of water metabolism in heart failure. *Adv. Exp. Med. Biol.* **449**, 415–426 (1998).
67. Ma, T. *et al.* Generation and phenotype of a transgenic knockout mouse lacking the mercurial-insensitive water channel aquaporin-4. *J. Clin. Invest.* **100**, 957–962 (1997).
68. Ma, T. *et al.* Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. *Proc. Natl Acad. Sci. USA* **97**, 4386–4391 (2000).
69. Roudier, N. *et al.* AQP3 deficiency in humans and the molecular basis of a novel blood group system, GIL. *J. Biol. Chem.* **277**, 45854–45859 (2002).
70. Nielsen, S., King, L. S., Christensen, B. M. & Agre, P. Aquaporins in complex tissues. II. Subcellular distribution in respiratory and glandular tissues of rat. *Am. J. Physiol.* **273**, C1549–C1561 (1997).
71. Kreda, S. M., Gynn, M. C., Fenstermacher, D. A., Boucher, R. C. & Gabriel, S. E. Expression and localization of epithelial aquaporins in the adult human lung. *Am. J. Respir. Cell Mol. Biol.* **24**, 224–234 (2001).
72. Krane, C. M. *et al.* Aquaporin 5-deficient mouse lungs are hyperresponsive to cholinergic stimulation. *Proc. Natl Acad. Sci. USA* **98**, 14114–14119 (2001).
73. King, L. S. & Agre, P. Man is not a rodent: aquaporins in the airways. *Am. J. Respir. Cell Mol. Biol.* **24**, 221–223 (2001).
74. Borok, Z. *et al.* Keratinocyte growth factor modulates alveolar epithelial cell phenotype *in vitro*: expression of aquaporin 5. *Am. J. Respir. Cell Mol. Biol.* **18**, 554–561 (1998).
75. King, L. S., Nielsen, S. & Agre, P. Aquaporin-1 water channel protein in lung: ontogeny, steroid-induced expression, and distribution in rat. *J. Clin. Invest.* **97**, 2183–2191 (1996).

76. King, L. S., Nielsen, S. & Agre, P. Aquaporins in complex tissues. I. Developmental patterns in respiratory and glandular tissues of rat. *Am. J. Physiol.* **273**, C1541–C1548 (1997).
77. Moon, C., King, L. S. & Agre, P. Aqp1 expression in erythroleukemia cells: genetic regulation of glucocorticoid and chemical induction. *Am. J. Physiol.* **273**, C1562–C1570 (1997).
78. Leitch, V., Agre, P. & King, L. S. Altered ubiquitination and stability of aquaporin-1 in hypertonic stress. *Proc. Natl Acad. Sci. USA* **98**, 2894–2898 (2001).
79. Towne, J. E., Krane, C. N., Bachurski, C. J. & Menon, A. G. Tumor necrosis factor-α inhibits aquaporin 5 expression in mouse lung epithelial cells. *J. Biol. Chem.* **276**, 18657–18664 (2001).
80. Yang, F., Kawedia, J. D. & Menon, A. G. Cyclic AMP regulates aquaporin 5 expression at both transcriptional and post-transcriptional levels through a protein kinase A pathway. *J. Biol. Chem.* **278**, 32173–32180 (2003).
81. Hoffert, J. D., Leitch, V., Agre, P. & King, L. S. Hypertonic induction of aquaporin-5 expression through an ERK-dependent pathway. *J. Biol. Chem.* **275**, 9070–9077 (2000).
82. Jayaraman, S., Song, Y., Vetrivel, L., Shankar, L. & Verkman, A. S. Noninvasive *in vivo* fluorescence measurement of airway-surface liquid depth, salt concentration, and pH. *J. Clin. Invest.* **107**, 317–324 (2001).
83. Hunt, J. F. *et al.* Endogenous airway acidification: implication for asthma pathophysiology. *Am. J. Respir. Crit. Care Med.* **161**, 694–699 (2000).
84. Ballard, S. T., Trout, L., Bebok, Z., Sorscher, E. J. & Crews, A. CFTR involvement in chloride, bicarbonate, and liquid secretion by airway submucosal glands. *Am. J. Physiol.* **277**, L694–L699 (1999).
85. Song, Y. & Verkman, A. S. Aquaporin-5 dependent fluid secretion in airway submucosal glands. *J. Biol. Chem.* **276**, 41288–41292 (2001).
86. James, A. L., Hogg, J. C., Dunn, L. A. & Pare, P. D. The use of the internal perimeter to compare airway size and to calculate smooth muscle shortening. *Am. Rev. Respir. Dis.* **138**, 136–139 (1988).
87. Anderson, S. D. & Daviskas, E. The mechanism of exercise-induced asthma is… *J. Allergy Clin. Immunol.* **106**, 453–459 (2000).
88. Bai, C. *et al.* Lung fluid transport in aquaporin-1 and aquaporin-4 knockout mice. *J. Clin. Invest.* **103**, 555–561 (1999).
89. King, L. S., Nielsen, S., Agre, P. & Brown, R. H. Decreased pulmonary vascular permeability in aquaporin-1-null humans. *Proc. Natl Acad. Sci. USA* **99**, 1059–1063 (2002).
90. Brown, R. H., Zerhouni, E. A. & Mitzner, W. Visualization of airway obstruction *in vivo* during pulmonary vascular engorgement and edema. *J. Appl. Physiol.* **78**, 1070–1078 (1995).
91. Dobbs, L. G. *et al.* Highly water-permeable type I alveolar epithelial cells confer high water permeability between the airspace and vasculature in rat lung. *Proc. Natl Acad. Sci. USA* **95**, 2991–2996 (1998).
92. Bai, C. *et al.* Lung fluid transport in aquaporin-1 and aquaporin-5 knockout mice. *J. Clin. Invest.* **103**, 555–561 (1999).
93. Ma, T., Fukuda, N., Song, Y., Matthay, M. A. & Verkman, A. S. Lung fluid transport in aquaporin-5 knockout mice. *J. Clin. Invest.* **105**, 93–100 (2000).
94. Towne, J. E., Harrod, K. S., Krane, C. M. & Menon, A. G. Decreased expression of aquaporin AQP1 and AQP5 in mouse lung after acute viral infection. *Am. J. Respir. Cell Mol. Biol.* **22**, 34–44 (2000).
95. Leikauf, G. D. *et al.* Pathogenomic mechanisms for particulate matter induction of acute lung injury and inflammation in mice. *Res. Resp. Health Eff. Inst.* **105**, 5–58 (2001).
96. Ma, T., Fukuda, N., Song, Y., Matthay, M. A. & Verkman, A. S. Lung fluid transport in aquaporin-5 knockout mice. *J. Clin. Invest.* **105**, 93–100 (2000).
97. Song, Y., *et al.* Role of aquaporins in alveolar fluid clearance in neonatal and adult lung, and in oedema formation following acute lung injury. *J. Physiol.* **525**, 771–779 (2000).
98. Hamann, S. *et al.* Aquaporins in complex tissues: distribution of aquaporins 1–5 in human and rat eye. *Am. J. Physiol.* **274**, C1332–C1345 (1998).
99. Mulders, S. M. *et al.* Water channel properties of major intrinsic protein of lens. *J. Biol. Chem.* **270**, 9010–9016 (1995).
100. Shiels, A. & Bassnett, S. Mutations in the founder of the MIP gene family underlie cataract development in the mouse. *Nature Genet.* **12**, 212–215 (1996).
101. Berry, V., Francis, P., Kaushal, S., Moore, A. & Bhattacharya, S. Missense mutations in MIP underlie autosomal dominant 'polymorphic' and lamellar cataracts linked to 12q. *Nature Genet.* **25**, 15–17 (2000).
102. Francis, P. *et al.* Functional impairment of lens aquaporin in two families with dominantly inherited cataracts. *Hum. Mol. Genet.* **9**, 2329–2334 (2000).
103. Thiagarajah, J. R. & Verkman, A. S. Aquaporin deletion in mice reduces corneal water permeability and delays restoration of transparency after swelling. *J. Biol. Chem.* **277**, 19139–19144 (2002).
104. Zhang, D., Vetrivel, L. & Verkman, A. S. Aquaporin deletion in mice reduces intraocular pressure and aqueous fluid production. *J. Gen. Physiol.* **119**, 561–569 (2002).
105. Raina, S., Preston, G. M., Guggino, W. B. & Agre, P. Molecular cloning and characterization of an aquaporin cDNA from salivary, lacrimal, and respiratory tissues. *J. Biol. Chem.* **270**, 1908–1912 (1995).
106. Gresz, V. *et al.* Identification and localization of aquaporin water channels in human salivary glands. *Am. J. Physiol.* **281**, G247–G254 (2001).
107. Steinfeld, S. *et al.* Abnormal distribution of aquaporin-5 water channel protein in salivary glands from Sjogren's syndrome patients. *Lab. Invest.* **81**, 143–148 (2001).
108. Tsubota, K., Hirai, S., King, L. S., Agre, P. & Ishida, N. Defective cellular trafficking of lacrimal gland aquaporin-5 in Sjogren's syndrome. *Lancet* **357**, 688–689 (2001).
109. Beroukas, D., Hiscock, J., Jonsson, R., Waterman, S. A. & Gordon, T. P. Subcellular distribution of aquaporin 5 in salivary glands in primary Sjogren's syndrome. *Lancet* **358**, 1875–1876 (2001).
110. Ma, T. *et al.* Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. *J. Biol. Chem.* **274**, 20071–20074 (1999).
111. Krane, C. M. *et al.* Salivary acinar cells from aquaporin 5-deficient mice have decreased membrane water permeability and altered cell volume regulation. *J. Biol. Chem.* **276**, 23413–23420 (2001).
112. Nejsum, L. M. *et al.* Functional requirement of aquaporin-5 in plasma membranes of sweat glands. *Proc. Natl Acad. Sci. USA* **99**, 511–516 (2002).
113. Song, Y., Sonawane, N. & Verkman, A. S. Localization of aquaporin-5 in sweat glands and functional analysis using knockout mice. *J. Physiol.* **54**, 561–568 (2002).
114. Moore, M., Ma, T., Yang, B. & Verkman, A. S. Tear secretion by lacrimal glands in transgenic mice lacking water channels AQP1, AQP3, AQP4, and AQP5. *Exp. Eye Res.* **70**, 557–562 (2000).
115. Umenishi, F. & Schrier, R. W. Hypertonicity-induced aquaporin-1 (AQP1) expression is mediated by the activation of MAPK pathway and hypertonicity-responsive element in the *AQP1* gene. *J. Biol. Chem.* **278**, 15765–15770 (2003).
116. Badaut, J., Lasbennes, F., Magistretti, P. J. & Regli, L. Aquaporins in brain: distribution, physiology, and pathophysiology. *J. Cereb. Blood Flow Metab.* **22**, 367–378 (2002).
117. Nielsen, S. *et al.* Specialized membrane domains for water transport in glial cells: high-resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J. Neurosci.* **17**, 171–180 (1997).
118. Amiry-Moghaddam, M. & Otterson, O. P. The molecular basis of water transport in the brain. *Nature Rev. Neurosci.* **4**, 991–1001 (2003).
119. Amiry-Moghaddam, M. *et al.* α syntrophin deletion removes the perivascular but not the endothelial pool of aquaporin-4 at the blood–brain barrier and delays the development of brain edema in an experimental model of acute hyponatremia. *FASEB J.* **18**, 542–544 (2004).
120. Neely, J. D. *et al.* Syntrophin-dependent expression and localization of aquaporin-4 water channel protein. *Proc. Natl Acad. Sci. USA* **98**, 14108–14113 (2001).
121. Hung, A. Y. & Sheng, M. PDZ domains: structural modules for protein complex assembly. *J. Biol. Chem.* **277**, 5699–5702 (2002).
122. Madrid, R. *et al.* Polarized trafficking and surface expression of the AQP4 water channel are coordinated by serial and regulated interactions with different clathrin-adaptor complexes. *EMBO J.* **20**, 7008–7021 (2001).
123. Monaco, A. P. *et al.* Isolation of candidate cDNAs for portions of the Duchenne muscular dystophy gene. *Nature* **323**, 646–650 (1986).
124. Frigeri, A. *et al.* Muscle loading modulates aquaporin-4 expression in skeletal muscle. *FASEB J.* **15**, 1282–1284 (2001).
125. Amiry-Moghadam, M. *et al.* An α-syntrophin dependent pool of AQP4 in astroglial end-feet confers bi-directional water flow between blood and brain. *Proc. Natl Acad. Sci. USA* **100**, 2106–2111 (2003).
126. Manley, G. T. *et al.* Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke. *Nature Med.* **6**, 159–163 (2000).

These researchers show that the absence of AQP4 in the brain is protective against oedema formation in two models of brain oedema.

127. Vajde, Z. *et al.* Delayed onset of brain edema and mislocalization of aquaporin-4 in dystrophin-null transgenic mice. *Proc. Natl Acad. Sci. USA* **99**, 13131–13136 (2002).
128. Nielsen, S., Smith, B. L., Christensen, E. I. & Agre, P. Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia. *Proc. Natl Acad. Sci. USA* **90**, 7275–7279 (1993).
129. Elkjaer, M. *et al.* Immunolocalization of AQP9 in liver, epididymis, testis, spleen, and brain. *Biochem. Biophys. Res. Commun.* **276**, 1118–1128 (2000).
130. Ma, T., Hara, M., Sougrat, R., Verbavatz, J.-M. & Verkman, A. S. Impaired stratum corneum hydration in mice lacking epidermal water channel aquaporin-3. *J. Biol. Chem.* **277**, 17147–17153 (2002).
131. Hara, M. & Verkman, A. S. Glycerol replacement corrects defective skin hydration, elasticity and barrier function in aquaporin-3-deficient mice. *Proc. Natl Acad. Sci. USA* **100**, 7360–7365 (2003).
132. Burghardt, B. *et al.* Distribution of aquaporin water channels AQP1 and AQP5 in the ductal system of the human pancreas. *Gut* **52**, 1008–1016 (2003).
133. Beitz, E., Kumagami, H., Krippeit-Drews, P., Ruppersberg, J. P. & Schultz, J. E. Expression pattern of aquaporin water channels in the inner ear of the rat. *Hear. Res.* **132**, 76–84 (1999).
134. Beitz, E., Zenner, H. P. & Schultz, J. E. Aquaporin-mediated fluid regulation in the inner ear. *Cell Mol. Neurobiol.* **23**, 315–329 (2003).
135. Li, J. & Verkman, A. S. Impaired hearing in mice lacking aquaporin-4 water channels. *J. Biol. Chem.* **276**, 31233–31237 (2001).
136. Richard, C., Gao, J., Brown, N. & Reese, J. Aquaporin water channel genes are differentially expressed and regulated by ovarian steroids during the periimplantation period in the mouse. *Endocrinology* **144**, 1533–1541 (2003).
137. Calamita, G., Mazzone, A., Bizzoca, A. & Svelto, M. Possible involvement of aquaporin-7 and -8 in rats testis development and spermatogenesis. *Biochem. Biophys. Res. Commun.* **288**, 619–625 (2001).
138. Kishida, K. *et al.* Aquaporin adipose, a putative glycerol channel in adipocytes. *J. Biol. Chem.* **275**, 20896–20902 (2000).
139. Kondo, H. *et al.* Human aquaporin adipose (*AQPao*) gene. Genomic structure, promoter analysis and functional mutation. *Eur. J. Biochem.* **269**, 1814–1826 (2002).
140. Carbrey, J. M. *et al.* Aquaglyceroporin AQP9: solute permeation and metabolic control of expression in liver. *Proc. Natl Acad. Sci. USA* **100**, 2945–2950 (2003).
141. Liu, Z. *et al.* Arsenite transport by mammalian aquaglyceroporins AQP7 and AQP9. *Proc. Natl Acad. Sci. USA* **99**, 6053–6058 (2002).
142. King, L. S. & Yasui, M. Aquaporins and disease: lessons from mice to humans. *Trends Endocrin. Metab.* **13**, 355–360 (2002).

Acknowledgements

The authors receive support from the National Heart, Lung and Blood Institute (L.S.K. and P.A.) and the National Eye Institute (P.A.) of the National Institutes of Health, USA, and from the American Heart Association (L.S.K.).

Competing interests statement

The authors declare no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:

Entrez: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi
AqpZ | GlpF
Interpro: http://www.ebi.ac.uk/interpro/
PDZ domain
OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
autosomal dominant NDI | autosomal recessive NDI | Sjögren's syndrome
Protein Data Bank: http://www.rcsb.org/pdb/
1J4N
Swiss-Prot: http://us.expasy.org/sprot/
AQP0 | AQP1 | AQP2 | AQP3 | AQP4 | AQP5 | AQP6 | AQP7 | AQP8 | AQP9 | AQP10

Access to this links box is available online.