Aquaporin-1 and Osmosis
From Physiology to Precision in Peritoneal Dialysis

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Abstract

The discovery of the aquaporin family of water channels has provided a molecular counterpart to the movement of water across biological membranes. The distribution of aquaporins in specific cell types, their selectivity and very high capacity for water permeation, and the control of their expression and/or trafficking are key to sustain osmosis in multiple tissues. Here, we review the convergent evidence demonstrating that aquaporin-1 (AQP1) facilitates water transport across endothelial cells in the peritoneal membrane, a key process for peritoneal dialysis—the leading modality of home-based dialysis therapy for patients with kidney failure. Genetic and pharmacologic studies in mouse and cell models indicated that AQP1 plays a critical role in crystalloid osmosis, with clinically relevant effects on water transport and risk of death and technique failure for patients on dialysis. By contrast, AQP1 plays no role in colloid osmosis. These studies substantiate potential strategies to improve free water transport and ultrafiltration in patients treated by peritoneal dialysis.

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In October 1826, the French physician Henri Dutrochet (1776–1847) presented to the Academy of Sciences in Paris a phenomenon that he believed was fundamental to biology. Based on careful microscopical observations, Dutrochet suggested that dense fluids filled within closed membrane systems are able to attract an inflow of water, causing visible swelling. He coined the term *endosmosis* for this inward transfer of water across the membrane. A simple experimental device, made by a piece of gut tied to form a bag over the end of a glass tube, supported the idea. When the bag was filled with a dense fluid, for instance, a sugar solution, and immersed in water, it was swelling—as predicted (Figure 1).[1] The concept of osmosis was born, referring to the movement of water across a barrier that is permeable to water but restricts the transport of solutes—which therefore act as osmotic agents. Osmosis is a fundamental force operating in all living organisms, for instance, in peritoneal dialysis (PD)—the leading form of home-based dialysis therapy for patients with kidney failure.[4]

In this brief review, we will discuss the concept of osmosis; its central relevance for PD; the cumulative evidence supporting the role of aquaporin-1 (AQP1), the first identified water channel, for water transport during PD; the role of AQP1 in crystalloid versus colloid osmosis; the biological and clinical effects of variants in the *AQP1* gene; and how this knowledge led to potential strategies to improve water transport and ultrafiltration in patients treated by PD.

Osmosis and Structure of the Cell Membrane

The work of Dutrochet in the 1820s suggested a possible mechanism for the direction of water movement across tissue, as evidenced by the gut membrane osmometer (Figure 1A). Dutrochet also used plant tissues in addition to animal samples to facilitate observation and translation into physiology. He observed the same inward movement of water in plants and when using a porous clay vessel, demonstrating the physical nature of osmosis.[5]

In 1861, the Scottish chemist Thomas Graham distinguished osmotic agents based on their physical properties: Small-sized osmotic agents prone to crystallization and easily diffusing across membranes were termed *crystalloid*, whereas substances unable to crystallize and slowly diffusing because of their larger size were referred to as *colloid*.[6] The properties of osmosis were further detailed by the German plant physiologist Wilhelm Pfeffer in his book *Osmotic Investigations* (1877), building on the observations of Carl Wilhelm von Nägeli who studied plant physiology in Zurich in the 1850s. Pfeffer's measurements were instrumental for the work of Jacob van't Hoff on solutions and osmotic pressure, for which he received the 1901 Nobel Prize in Chemistry.[7]

The studies of Charles Ernest Overton on the structure and function of the cell membrane, performed from 1884 to 1901 in Zurich, completed the concept of osmosis.[2] Using plant cells, Overton established that a lipid-impregnated boundary layer, possibly involving phospholipids and

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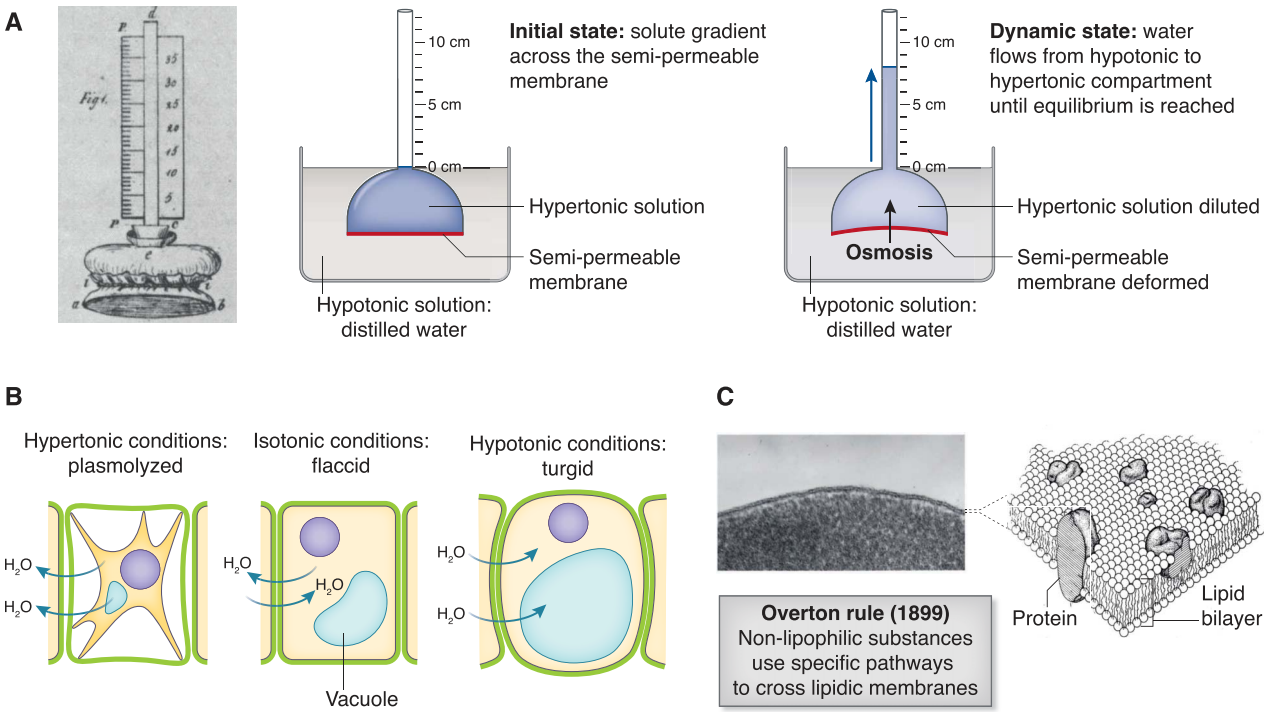

Figure 1. Osmosis and structure–function relationship in the cell membrane. (A) The first osmometer conceived by Dutrochet (1826). The device allowed to demonstrate the movement of water across a semipermeable membrane, causing a dilution of the hypertonic solution contained in the recipient and the gradual move of fluid upward in the tube. (B) The phenomenon of plasmolysis in plant cells, due to osmotically induced flows of water across the cell membrane, exposed to various solutions. The system was used by Overton (1899) to investigate the osmotic properties of the cell membrane. (C) The concepts of Overton, for example, lipid boundaries and pathways for solute transport, were integrated in the Singer and Nicolson fluid mosaic model of the structure of the cell membrane (1972). Modified from refs. 1–3, with permission.

cholesterol, is a determinant of the osmotic properties of living cells (Figure 1, B and C). By testing hundreds of different solutes, he recognized the relationship between the chemical structures of solutes and their osmotic properties. Lipid-soluble solutes, such as anesthetics or ethanol, penetrated the cell membrane fastest, whereas physiological solutes, like glucose or amino acids or inorganic salts, did not seem to diffuse across the cell membrane. Thus, the determinant for a solute passing the cell membrane barrier is its solubility in the membrane, which led Overton to postulate the existence of pathways by which solutes can cross the osmotic barrier.

The hypothesis of Overton has sustained the test of time and is included in the fluid mosaic model of cell membranes proposed by Singer and Nicolson in 1972.[3] In this model, membranes are composed of a lipid bilayer with proteins mediating the exchange of solutes between cells and their immediate environment (Figure 1C). Large-scale analyses of these transport proteins, which operate in all living cells, on the basis of DNA sequencing, bioinformatic analyses, and experimental characterization are now possible.[8] To date, 1467 transporters have been identified in *Homo sapiens*, with characteristic distribution, substrate specificity, and modes of activation and regulation (http://www.membranetransport.org/transportDB2/index.html). This level of complexity is reflected by the fact that membrane transporters mediate the transport of most physiologically relevant molecules, whatever their lipidic solubility.[9]

PD: Osmosis in Action

One of the most obvious applications of osmosis in medicine is the dialysis procedure, used to treat patients with kidney failure. CKD affects >800 million individuals and is one of the most prominent causes of mortality worldwide. The global prevalence of CKD increased by 33% between 1990 and 2017, and the burden of CKD among leading causes of death is continuously raising.[10] In 2010, approximately 2.6 million people received KRT worldwide, and this number is estimated to double to 5.4 million by 2030.[11] Still, worldwide, millions of people die of kidney failure each year because of the lack of access to KRT. Current recommendations for kidney care programs, particularly in low- and middle-income countries, put emphasis on treatments to prevent or delay kidney failure, conservative care, living-donor kidney transplantation, and PD.[11–13]

PD is the leading form of home-based dialysis therapy for patients with kidney failure, with advantages that include simplicity, lower need for trained medical staff, minimal technical support, lower costs, and access in remote and rural locations (Figure 2).[15] These features explain why PD has a major potential to improve access

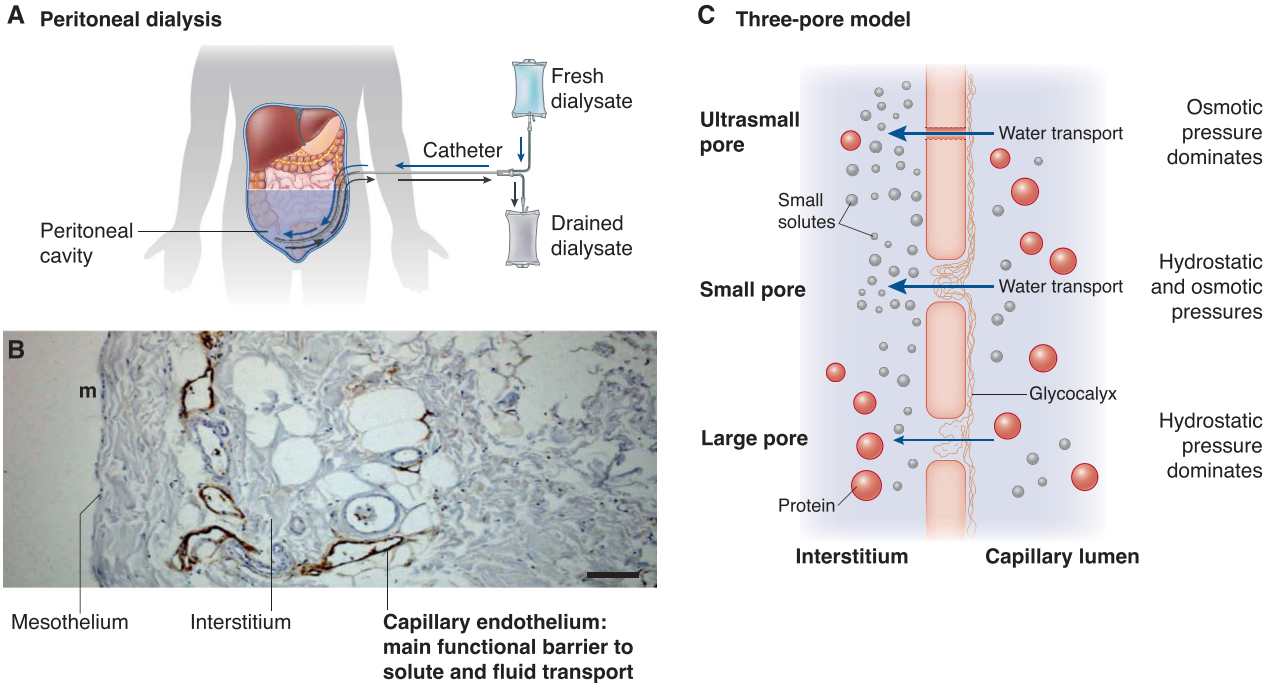

Figure 2. Principle of dialysis across the peritoneal membrane. (A) PD applies the principle of osmosis across the peritoneal membrane to remove water and solutes accumulated in patients with kidney failure. To perform PD, a fresh dialysate fluid is inserted into the peritoneal cavity through a catheter. During the dwell, the dialysate absorbs the waste, toxins, and excess fluid from blood, to be drained out of the peritoneal cavity at the end of the exchange. Ultrafiltration is chiefly driven by osmotic gradients and influenced by hydrostatic pressure. (B) Peritoneal membrane including a dense capillary network with the endothelium immunostained for factor VIII. Bar: 100 μm. (C) The three-pore model for the transport of water and solutes across the capillary endothelium during PD. The model predicted the existence of ultrasmall pores that facilitate water transport when using hypertonic glucose dialysate for PD. Modified from refs. 4 and 14, with permission. m, mesothelium; PD, peritoneal dialysis; UF, ultrafiltration.

to dialysis, particularly in vulnerable populations and lower-resource countries.[13] The technique of PD for long-term treatment of uremia was initiated in the late 1950s, with subsequent development of the indwelling catheter providing easy access to the peritoneal cavity and standardization of the glucose-based dialysates leading to continuous ambulatory PD. Improvements in the quality of fluids, availability of alternate osmotic agents, better connectivity, and development of automated PD have further improved dialysis efficacy and quality of life of the treated patients.[14,15] Globally, PD accounts for an approximately 11% of patients receiving long-term dialysis worldwide,[11] with a prevalence increasing substantially in many parts of the world.[16]

The principles governing solute and fluid transport across the peritoneal membrane during PD are diffusion, driven by a concentration gradient, and convection, that is, ultrafiltration chiefly driven by osmotic gradients and influenced by hydrostatic pressure (Figure 2).[4] The efficiency of PD depends on its ability to remove excess of water and to restore normal body fluid status, as well as to clear waste substances.[4] Functional alterations in the dialysis capacity of the membrane, leading to ultrafiltration failure and fluid overload, are associated with higher morbidity and mortality and represent a major obstacle to successful long-term PD therapy.[14,15] The peritoneal membrane is a thin, translucent membrane covering the visceral organs and the inner surface of the abdominal wall, delineating the peritoneal cavity (Figure 2B). The membrane (surface, approximately 1 m^2) contains a high density of capillaries, receiving a 100–150 ml/min blood flow in adults, distributed in an interstitium. The peritoneal membrane is lined by a mesothelium, which reduces the friction between visceral organs.

Bengt Rippe and colleagues described the transport of water and solutes across the endothelium lining peritoneal capillaries during PD in a three-pore model.[17,18] The three-pore model includes (*1*) numerous small pores located at the interendothelial clefts, mediating solute-coupled fluid transport, (*2*) ultrasmall pores located in endothelial cells, facilitating transcellular water transport, and (*3*) a relatively small number of large pores, accounting for the transport of macromolecules (*e.g.*, albumin, immunoglobulins) with no role in water transport (Figure 2C). Notably, the three-pore model predicted that the ultrasmall water pores should have a functional radius of approximately 2.5 Å and would account for up to 50% of ultrafiltration achieved when using hypertonic glucose dialysate for PD. Their ability to mediate fast water transport would also explain the rapid dilution of dialysate sodium concentration during the first part of the dwell (denoted sodium sieving).[4,18] Identification of the molecular counterpart of these ultrasmall pores was critical for understanding water transport and developing strategies to reduce complications and improve outcome of PD.

AQP1: A Water Channel Expressed in Endothelial Cells

The discovery and characterization by Peter Agre of AQP1 as the first member of the aquaporin water channel family explained how water can specifically flow across cell membranes, a process of major importance in physiology and medicine (Figure 3).[19] For this work, Agre received the 2003 Nobel Prize in Chemistry, a century after that awarded to van't Hoff.

AQP1 is a 28-kD integral membrane protein, first identified in human erythrocytes and kidney, where it was localized in the proximal tubules and thin descending limbs of the loop of Henle.[22] Biochemical studies of the 28-kD protein from erythrocytes indicated that it exists as a tetramer and bears homology and membrane organization similar to MIP26, the major intrinsic protein of the lens, and other members of a newly identified family of membrane channels.[23] The protein was named CHIP28 (channel-forming integral protein of 28 kD) and its cDNA cloned, predicting six transmembrane domains organized in two tandem repeats, two conserved Asn-Pro-Ala motifs, and intracellular NH2-termini and COOH-termini (Figure 3B).[24] The homology between CHIP28 and potential channels linked to water permeability, and its specific distribution in kidney tubular segments that are constitutively permeable to water, led Agre, Guggino, and colleagues to investigate whether CHIP28 was indeed a water channel—using the osmotic swelling assay in oocytes from *Xenopus laevis*.[20] In this famous experiment, control-injected oocytes placed in hypotonic

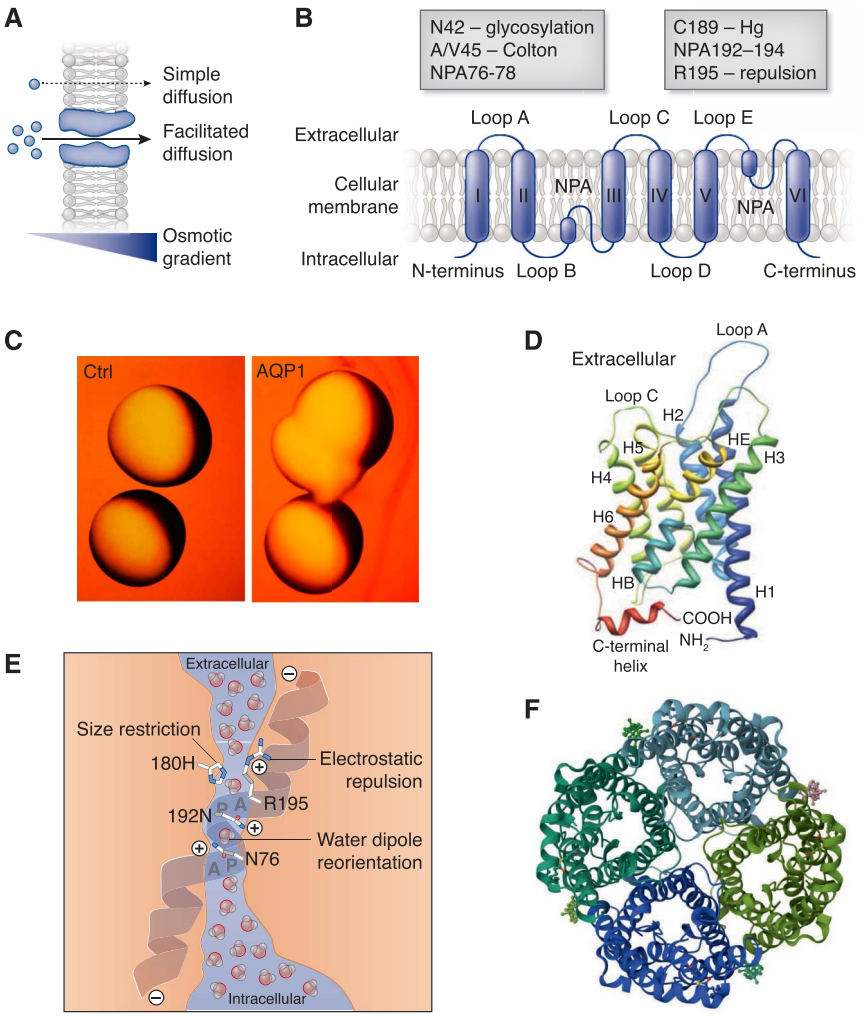

Figure 3. AQP1 water channel. (A) Simple or channel-mediated diffusion of water across cell membrane, driven by an osmotic gradient. (B) Membrane topology of the AQP1 subunit, with six transmembrane domains connected by five loops. The two conserved NPA motifs are folding back in the membrane and located in short pore helix participating in the water pore. Functionally important residues are boxed above the structure. (C) The osmotic swelling assay in oocytes from *Xenopus laevis*: Control-injected oocytes placed in hypotonic (70 mOsm) solution swelled minimally, whereas oocytes injected with RNA coding for AQP1 (then called CHIP28) consistently swelled up to 1.5-fold their initial volume and ruptured within minutes. (D) Structure of an AQP1 monomer, side view. The membrane-spanning helices are denoted as H1–H6, loops are denoted as A–E, and the two pore helices formed by loops B and E as HB and HE, respectively. (E) Key structural features involved in the selectivity of the water pore in AQP1. (F) Homotetrameric assembly of AQP1, front view. The structure (is for protein data bank entry 8ct2) contains four copies of AQP1 and four copies of cholesterol. Modified from refs. 19–21, with permission. AQP1, aquaporin-1; NPA, Asn-Pro-Ala.

solution swelled minimally and failed to rupture even after incubations >1 hour, whereas oocytes injected with CHIP28 RNA consistently swelled up to 1.5-fold their initial volume and ruptured within 5 minutes (Figure 3C). The changes were reversibly inhibited by $HgCl_2$, a known inhibitor of the osmotic water permeability. Altogether, these seminal observations supported that CHIP28 is the constitutive water channel operating in erythrocytes and kidney tubules.[20] The subsequent discovery of the vasopressin-activated water channel (aquaporin-2) operating in the collecting duct,[25] and of the γ-Tonoplast intrinsic protein operating in *Arabidopsis thaliana*,[26] prompted the renaming of CHIP28 into AQP1, the first functionally defined water-channel protein.[27]

AQP1 is distributed in many different cell types, including endothelial cells lining capillaries in the peritoneal membrane.[28,29] Each AQP1 monomer contains six membrane-spanning α-helices forming a right-handed twisted arrangement surrounding a hourglass-shaped central pore (Figure 3, D–F). Key structural features of AQP1 account for the high permeation rate (3 billion water molecules per subunit per second) and the selectivity of the channel for water.[19] The AQP1 pore (approximately 18-Å length) is lined with hydrophobic residues and has a narrow constriction of 2.8 Å, just sufficient to accommodate a single file of water molecules. Protons are reflected out of the pore by the electrostatic repulsion created by the positively charged arginine (R195) located at the narrowest site of the pore, completed by a conserved histidine (H180) on the other wall, and a transient reorientation of the water dipole as it forms hydrogen bonds with the side chains of the two conserved asparagine residues (N76 and N192) of the Asn-Pro-Ala motifs. This mechanism is breaking the alignment of hydrogen-bonded water molecules through the pore, thus preventing the transport of protons. In addition, two partial positive charges at the distal end of the nonbilayer spanning α-helices participate in the barrier to protons. The location of the side chain of a cysteine residue (C189) in the pore explains the inhibition of water transport by mercury compounds.

Although AQP1 is generally considered to function as a constitutive water channel, it can also be regulated by translocation, as indicated by the rapid changes in localization of AQP1 to the plasma membrane observed in primary cholangiocytes, astrocytes, and various cell lines, in response to various stimuli.[30,31]

AQP1 Is Crucial for Water Transport during PD

A series of evidence supports the crucial importance of AQP1 for water transport in the context of PD (Figure 4). The size of the AQP1 pore perfectly fits the postulated size of the ultrasmall pore of the peritoneal membrane.[4] AQP1 is the most abundant AQP isoform in the peritoneum, and the only one that has been consistently located in the capillary endothelium, the functional barrier of the peritoneal membrane (Figure 4A).[32,34] The use of the AQP1 knockout (KO) mice developed by Ma *et al.*[35] allowed to show that osmotically driven water transport across the peritoneal membrane is significantly decreased in *Aqp1* KO mice, as compared with wild-type littermates (Figure 4B).[36] Using a standard peritoneal exchange test, the *Aqp1* KO mice had (*1*) an approximately 50% decrease in ultrafiltration; (*2*) significantly decreased changes in intraperitoneal volumes; and (*3*) no evidence for sodium sieving compared with wild-type littermates—confirming the predictions of the three-pore model.[32] Heterozygous $Aqp1^{+/-}$ mice showed an intermediate phenotype, indicating that haploinsufficiency in AQP1 is sufficient to attenuate water transport.[32]

The role of AQP1 on water transport in the context of crystalloid osmosis in PD has been confirmed by using highly sensitive measurements of intraperitoneal volume and osmotic water transport[37] and in an endothelial-specific, inducible AQP1 KO mouse model.[38] Importantly, the deletion of AQP1 in these models had no effect on the structure of the peritoneal membrane, the magnitude of small solute transport, and the osmotic gradient.[32,38] The lack of any compensatory change in the peritoneal membrane of the *Aqp1* KO mice may indicate a relatively minor role of facilitated water transport in physiological conditions, that is, absence of osmotic gradient in the peritoneal cavity.

The expression of AQP1 may be induced by corticosteroids because of the presence of glucocorticoid response elements in the promoter of *AQP1*.[39,40] Accordingly, treatment of rats with dexamethasone (1–4 mg/kg BW) for 5 days significantly increased the expression of AQP1 in peritoneal capillaries, reflected by a significantly higher water transport and net ultrafiltration across the membrane.[41] The effect of corticosteroids on AQP1-mediated water transport was confirmed in PD patients that received high-dose glucocorticoids (cumulative dose of methylprednisolone: 1.0–1.2 g/m^2) as standard care after a living-donor kidney transplantation.[42]

Pharmacological Regulation of AQP1

Beyond the induction of aquaporin expression, pharmacological agonists may facilitate water transport by gating the water pore.[43] The lens water channel AQP0 and plant aquaporins show changes in water permeability as a function of pH.[44] Intracellular domains have been proposed to gate channel functions in AQP1[45,46] and in a plant aquaporin.[47] In the latter model, loop D is a hydrophobic barrier that caps and occludes the intracellular side of the water pore, in response to dephosphorylation of conserved serine residues or, alternatively, to the protonation of a conserved histidine (flooding stress).[47] Thus, stabilization of the open-state conformation of the pore by ligand binding would yield an agonist activity.

Modulators of AQP1 include a derivative of the loop diuretic bumetanide (AqB013), which was shown to inhibit AQP1 by acting at an intracellular side that occludes the water pore.[48] Docking simulations have identified a potential intracellular pocket that involves residues from the C-terminus and intracellular loop, also associated with regulation of AQP1 by protein kinase C.[46,48] A derivative of furosemide (AqF026) has been identified as an agonist of AQP1.[49] In the *Xenopus laevis* oocyte system, extracellularly applied AqF026 (5–20 μM) potentiated water channel activity of AQP1 by more than 20%. The intracellular binding site for AQP1, tested by

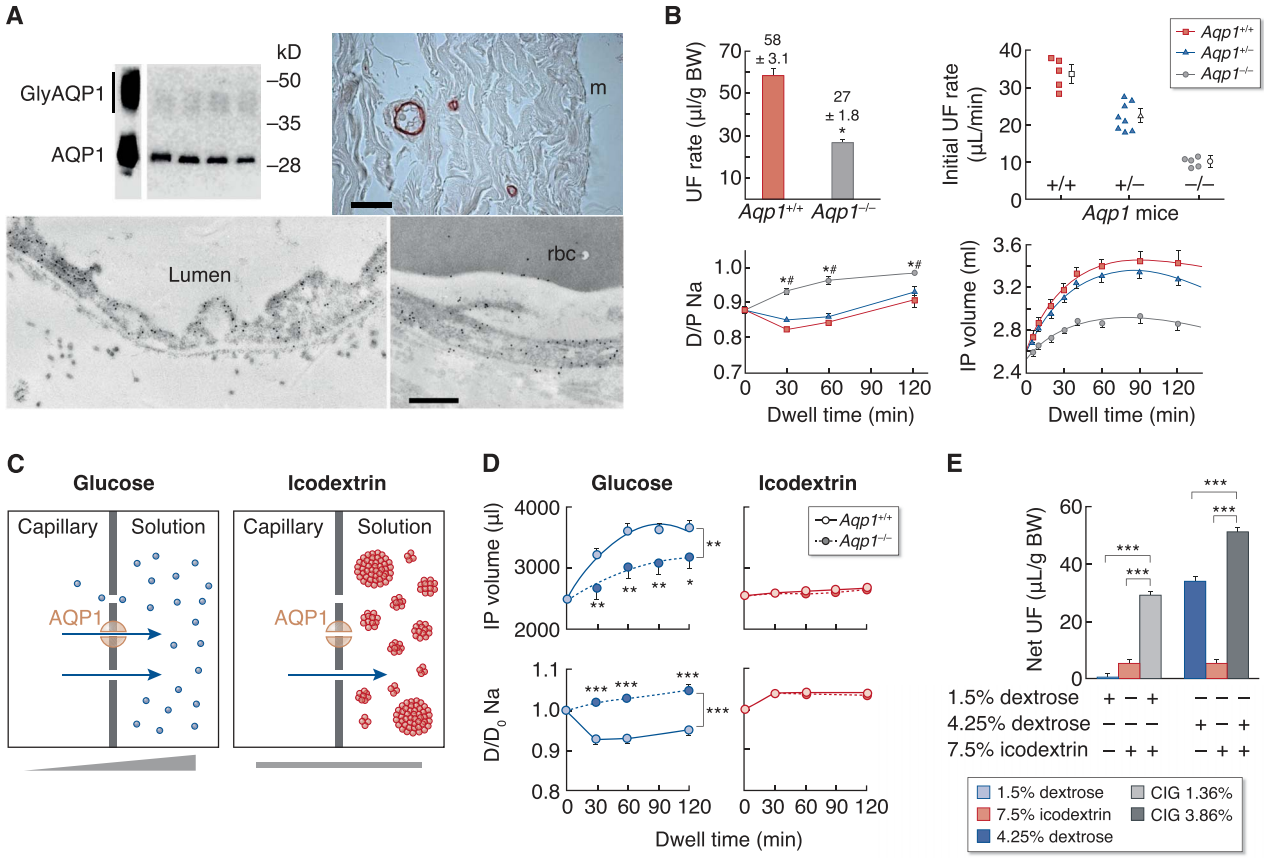

Figure 4. AQP1 and water transport in PD. (A) Expression and distribution of AQP1 in the peritoneal membrane, assessed by Western blot, immunostaining (human peritoneum, bar: 40 μM), and immunogold electron microscopy (mouse peritoneum, bar: 0.5 μM). AQP1 is highly expressed in the endothelial cells lining the peritoneal capillaries, venules, and small veins. (B) Effect of AQP1 gene dosage of ultrafiltration, initial ultrafiltration rate, and D/P sodium concentrations and IP volume over time in *Aqp1* mouse littermates exposed to standard peritoneal equilibration test using 3.86% glucose. The deletion of AQP1 reduces water transport and IP volume and abolishes the sodium sieving. $*P < 0.05$ versus *Aqp1*$^{+/+}$ mice; $\#P < 0.05$ versus *Aqp1*$^{+/-}$ mice. (C) Principles of crystalloid (glucose) and colloid (icodextrin) osmosis according to the tonicity gradient. (D) Effect of AQP1 deletion on IP volume and sodium sieving (D/D_0 Na^+), using glucose- or icodextrin-based dialysate. The hypertonic glucose-based dialysate induced a rapid increase in IP volume and a dialysate sodium sieving during the first part of the dwell in presence of AQP1. The deletion of AQP1 induces a significant reduction in the osmotic water permeability of the peritoneal membrane and the abolition of sodium sieving. Isotonic solutions containing icodextrin induced a slow and sustained ultrafiltration, with no sodium sieving; these kinetics are essentially unchanged in absence of AQP1. (E) Combination of crystalloid and colloid osmotic agents in the same dialysis solution enhance water transport across the peritoneal membrane, improving ultrafiltration efficiency over that obtained with either type of agent alone. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Modified from refs. 32 and 33, with permission. CIG, combined icodextrin and glucose; D/P, dialysate over plasma; IP, intraperitoneal; rbc, red blood cell.

site-directed mutagenesis and swelling assays, involves the loop D. *In vivo* assays in a mouse model of PD confirmed that AqF026 enhanced the osmotic water transport across the peritoneal membrane without detectable effect on the transport of small solutes, nor on the expression and/or plasma membrane localization of AQP1. AqF026 had no effect in *Aqp1*-null mice, arguing against indirect mechanisms involving other channels.[49]

Role of AQP1 in Crystalloid versus Colloid Osmosis

Glucose has long been used as the prototypic crystalloid osmotic agent to drive water removal in PD. However, the use of glucose is limited by its fast reabsorption, dissipating the osmotic gradient and inducing adverse metabolic effects, and its role in structural and functional alterations of the peritoneal membrane, including vascular proliferation and fibrotic changes.[4,14,15] These limitations motivated the search for alternative osmotic agents, including colloidal substances, such as icodextrin, an isotonic mixture of glucose polymers. Thanks to their larger hydrodynamic radius, polymers of icodextrin are absorbed from the peritoneal cavity at a much slower rate than glucose, making them particularly suitable for long dwells and sustained ultrafiltration (Figure 4C).[50,51]

The principles of crystalloid and colloid osmosis and the differential role of AQP1 in these processes have been substantiated recently in mouse models (Figure 4, C and D).[33] Hypertonic crystalloid dialysates induced a fast increase in intraperitoneal volume, a dialysate sodium

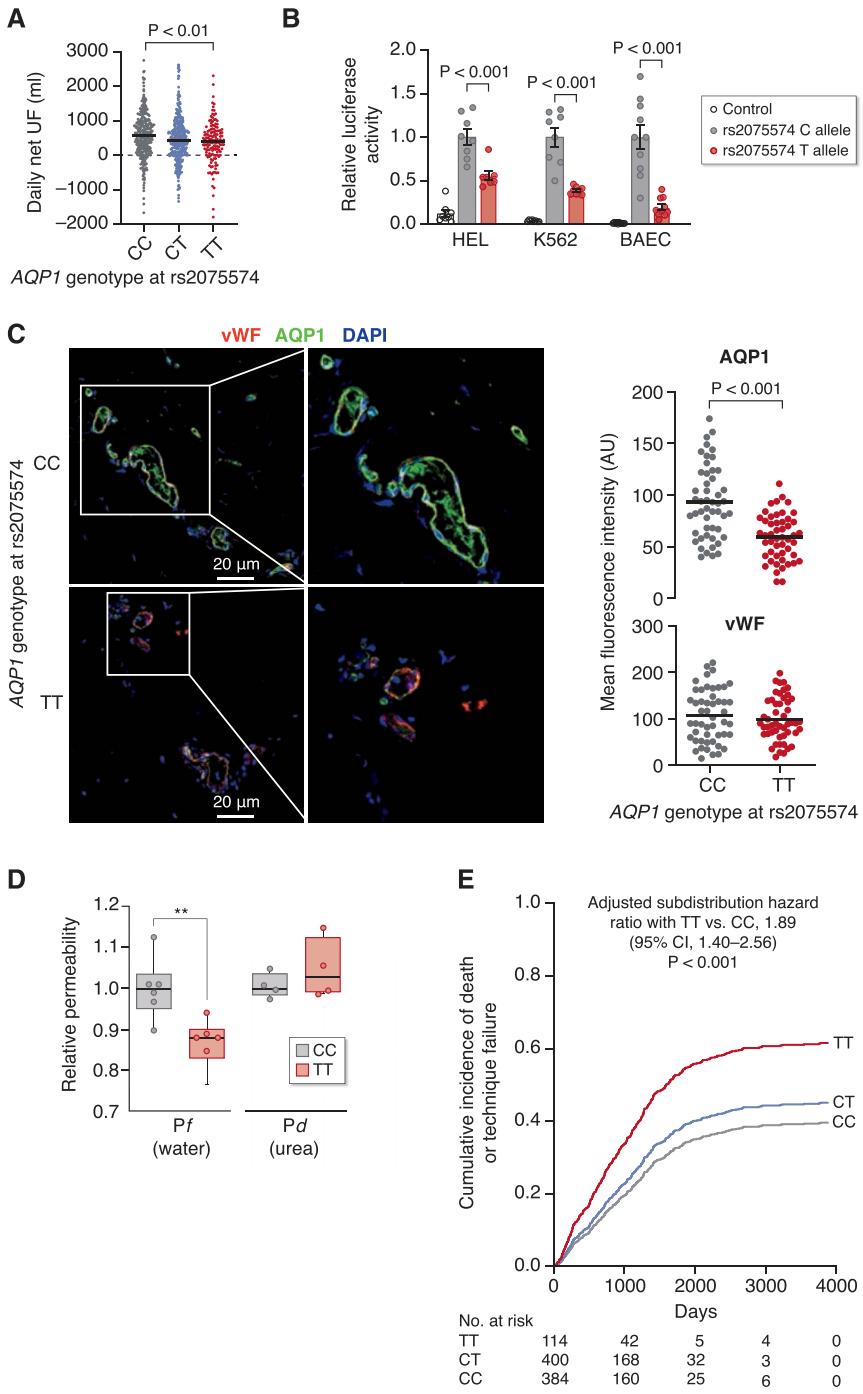

Figure 5. *AQP1* promoter variant, water transport, and outcomes in PD. (A) The rs2075574 (c.-781C>T) promoter variant of *AQP1* is associated with water transport (assessed by ultrafiltration) in 985 patients from three independent cohorts. Patients with the TT genotype had a significantly lower daily net ultrafiltration as compared with those with the CC genotype, with no changes in small solute transport nor residual diuresis. (B) Quantitative analysis of the relative effects of the major (C) and minor (T) alleles of rs2075574 on the transcriptional activity of the *AQP1* promoter as assessed by a luciferase reporter assay in HEL, K562, and BAEC cell types. Control corresponds to the promoter-less vector. Data result from four independent experiments and are expressed as means±SEM. (C) Endothelial expression of AQP1 quantified using confocal microscopy in peritoneal vessels from patients stratified for genotype at rs2075574. Representative images of double immunostaining with anti-AQP1 (green channel) and anti-vWF (red channel) antibodies. Nuclei are counterstained in blue with 4′,6-diamidino-2-phenylindole. Bar: 20 μm. Scatter plots show individual data and means. (D) Calculated osmotic permeability coefficients (P*f*) and permeabilities to urea (P*d*) in human red blood cells stratified for the *AQP1* risk variant. Box and whiskers show minimal to maximal and individual values; $n=6$ per group for osmotic water permeability and $n=4$ per group for urea permeability. $^{**}P = 0.006$ (unpaired *t* test). A significant reduction in the specific permeability for water is observed in red blood cell membranes from patients with TT compared with those with CC genotype. (E) The *AQP1* promoter risk variant is

Figure 5. *Continued.* associated with poor outcome in incident patients starting PD. Data were available for 898 participants. After a mean follow-up of 944 days, patients with the TT genotype showed a higher incidence of the composite of death or technique failure (58% versus 42%, respectively, $P = 0.01$) and a higher incidence of all-cause death (24% versus 15%, respectively, $P = 0.03$) compared with patients with the CC genotype. Modified from ref. 62, with permission. BAEC, bovine aortic endothelial; CI, confidence interval; HEL, human erythroleukemia; K562, human erythroleukemia; vWF, von Willebrand factor.

sieving, and a net ultrafiltration in wild-type mice, that were significantly reduced or abolished (sodium sieving) in *Aqp1* KO littermates. Conversely, isotonic icodextrin solutions induced a slow and sustained ultrafiltration, with no sodium sieving, and these characteristics were essentially unchanged in *Aqp1* KO mice. Contrasting with the transient osmotic effect of glucose, the water flow induced by icodextrin was constant over time, independent of the osmotic gradient, occurring despite the lower tonicity of the dialysate compared with that of plasma. Altogether, these studies demonstrated that icodextrin-induced osmosis occurs independently of water channels and tonicity.[33]

These investigations also provided evidence for the potential advantage of combining crystalloid and colloid agents in PD (Figure 4E).[52] The use of combined solutions led to a fast, glucose-induced, and AQP1-dependent osmotic water transport during the first part of the dwell and a sustained ultrafiltration thanks to the colloidal fractions of icodextrin. These colloidal fractions enhanced solute-coupled water removal and prevented the backflow of water from dialysate to peritoneal capillaries after dissipation of the crystalloid osmotic gradient. The enhanced ultrafiltration achieved using such combinations diluted glucose levels in the dialysate, thereby reducing its systemic absorption.[33] Combining crystalloid and colloid osmosis offers the potential to enhance water transport and achieve euvolemia—particularly in patients with fast peritoneal solute transport rate causing a loss of ultrafiltration capacity.[53]

Genetic Variants in AQP1 Influence Water Transport and Outcome in Dialysis

There is a large variability in solute and water transport across the peritoneal membrane in patients starting PD, influencing dialysis prescription and outcome.[54] Recent studies indicated a high heritability for peritoneal solute transport rate,[55] supporting a genetic contribution to individual peritoneal transport.[56] Studies in *Aqp1* mice have demonstrated that haploinsufficiency in AQP1 is sufficient to detect significant changes in osmotic water transport.[32,36,57] No specific disease has been linked to pathogenic variants in the *AQP1* gene in humans. Extremely rare persons who lack Colton antigens (Colton-null), because of a polymorphism at residue 45 located on the first extracellular loop of AQP1,[58] were found to be homozygous for nonsense and missense mutations in *AQP1* that resulted in a nonfunctioning water channel.[59] None of these persons presented any apparent clinical manifestations. Yet, under stress, the Colton-null individuals lacking AQP1 showed decreased fluid transport in the lung, and defective urinary concentration ability attributed to impaired water transport in the vasa recta.[60,61] These studies led us to test whether genetic variation in AQP1 may influence water transport and outcome in patients treated with PD (Figure 5).[62]

We gathered clinical and genetic data in 1851 PD patients from seven cohorts to screen whether variants in the *AQP1* gene associated with peritoneal water transport. Genotyping was obtained for common variants covering the major linkage disequilibrium blocks over *AQP1*. The rs2075574 *AQP1* promoter variant (c.-781C>T) was associated with water transport in PD. The TT carriers (10%–16% of patients) showed defective peritoneal water transport both in discovery and validation cohorts, compared with CC carriers (Figure 5A). Mechanistically, the rs2075574 variant is located in a conserved region of the *AQP1* promoter, corresponding to a transcription factor binding site (CTGTC) regulating the expression of erythroid-specific genes. The rs2075574 is a highly significant cis-eQTL in the *AQP1* locus.[63] The rs2075574 risk variant decreased *AQP1* promoter activity in endothelial cells lines (Figure 5B) and was associated with significantly decreased levels of AQP1 in peritoneal biopsies (Figure 5C). An approximately 50% decrease in expression of AQP1 was further documented in red blood cell membranes from patients with TT compared with those with CC genotype, reflected by a significant reduction in their specific permeability for water (Figure 5D). After a mean follow-up of 944 days, carrying the TT genotype was associated with higher risk for the composite of death and technique failure and a significantly higher proportion of all-cause deaths compared with the CC genotype (Figure 5E).[62]

In contrast to glucose-driven crystalloid osmosis, the water flow generated by the colloid osmotic agent icodextrin is independent of AQP1 (Figure 4C).[33] Notably, water transport generated by icodextrin was unchanged in the heterozygous *Aqp1*$^{+/-}$ mice, confirming the potential value of colloid agents for patients with a deleterious *AQP1* genotype. We tested whether the effect of rs2075574 variant on net ultrafiltration would be dependent of the type of osmotic agent in incident PD patients. Patients carrying the rs2075574 TT genotype showed a significant decrease in net ultrafiltration generated by hypertonic glucose, as compared with the CC patients. By contrast, there was no association between the rs2075574 variant and colloid osmosis induced by icodextrin.[62]

Together, these data demonstrate that the rs2075574 *AQP1* promoter variant influences osmotic water transport in patients treated by PD and is independently associated with a higher risk for death or technique failure. The rs2075574 variant influences the *AQP1* promoter activity, the expression of AQP1 in erythrocytes and peritoneal microvessels, and osmotic water permeability. The

effect of the *AQP1* genotype reflects a gene–environment interaction, as it requires exposure to a crystalloid osmotic gradient across the peritoneal membrane.[64] These results substantiate the influence of genetic factors on PD efficiency and open a perspective for precision medicine in dialysis.[65]

Conclusions and Perspectives

Translating physiological knowledge into molecular and genetic insights fills a need for precision medicine in the field of dialysis.[12,66] The discovery of the crucial role of AQP1 in mediating water transport across peritoneal capillaries provided a molecular counterpart to models of transport and osmosis during PD. It also yielded potential strategies to reduce complications and improve outcomes for the increasing number of patients treated with PD globally. Prospective studies, sufficiently powered and including patients from diverse ethnic origins, will need to test the predictive value of the *AQP1* variant and whether practices or adaptation of PD prescription may mitigate the risk conferred by this or other variants in *AQP1*. The value of potential interactions between *AQP1* variants and emerging polygenic risk scores for peritoneal transport will have to be investigated.[55]

The link between *AQP1* genotype and all-cause mortality in PD patients should require further attention. A lower expression of AQP1 in capillaries may lead to overhydration, promoting cardiovascular events, inflammation, and malnutrition.[60] The involvement of AQP1 in cell migration and angiogenesis,[67] regulation of macrophage motility,[68] and myocardial remodeling,[69] may also be considered. Increasing evidence also suggests that AQP1 facilitates the transport of nonwater solutes and gases, including H_2O_2, O_2, and CO_2, regulates intracellular and intercellular signaling, and interacts with junctional proteins.[21] The availability of large databases and resources (such UK Biobank and All of US) will offer opportunities to document the potential consequences of genetic variation in *AQP1*. A better understanding of these associations, and of the regulation of AQP1 in endothelial cells, will be important to pursue the search for small-molecule modulators of aquaporins.[70]

Finally, the remarkable properties of aquaporins to mediate fast and selective water transport inspired efforts to bridge disciplines and to design artificial water channels.[71–73] Such artificial water channels are needed to develop efficient and affordable biomimetic membranes used for water purification and desalination or to increase water transport and ultrafiltration in wearable, bioartificial kidneys.[74]

Disclosures

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