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REVIEW

PHYSIOLOGY AND PATHOPHYSIOLOGY OF AQUAPORINS

Amaia Rodríguez[1,4], Victoria Catalán[1,4], Sara Becerril[1,4], Andoni Lancha[1,4], Beatriz Ramírez[1,4], Javier Salvador[2,4], Javier Gómez-Ambrosi[1,4], Giovanna Valenti[3], Giuseppe Calamita[3], and Gema Frühbeck[1,2,4]

[1]Metabolic Research Laboratory and [2]Department of Endocrinology, Clínica Universidad de Navarra, University of Navarra, Pamplona, Spain; [3]Department of General and Environmental Physiology, University of Bari Aldo Moro, Bari, Italy; [4]CIBER Fisiopatología de la Obesidad y Nutrición, Instituto de Salud Carlos III, Spain.

Abstract

Aquaporins (AQPs) are water channels that facilitate a rapid transport of water, across cell membranes. In some cases, these pores are also permeated by small solutes, particularly glycerol. Thirteen aquaporins (AQP0-12) have been identified so far in mammalian tissues. The disruption of the genes encoding aquaporins in transgenic mice has revealed their implication in physiological and pathophysiological processes, including renal water absorption, neural function, digestion, tumour angiogenesis, and reproduction. A subset of aquaporins that transport both water and glycerol, the 'aquaglyceroporins', regulate glycerol content in epidermal, fat and other tissues, and are involved in skin hydration, fat metabolism and gluconeogenesis. Better understanding of the exact mechanisms and regulation of aquaporins might be useful for designing potential drug targets against different metabolic disorders, such as stroke, glaucoma, brain ooedema, cancer, diabetes and obesity.

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Introduction

Aquaporins are channel-forming integral membrane proteins that allow the movement of water through cell membranes (1). The secondary structure proposed for aquaporins predicted six bilayer-spanning domains and two asparagine-proline-alanine motifs (NPA boxes) that confer selectivity for water and/or other solutes (2) (Fig. 1). The three-dimensional structure of aquaporin resembles an hourglass; within the lipid belayed aquaporins usually form tetramers, with each monomer defining a single pore. To date, 13 members of the family of aquaporins (AQP0-12) have been identified in different mammalian tissues. According to their permeability characteristics, aquaporins can be divided into two subgroups: aquaporins (pure water channels) and aquaglyceroporins (channels permeated by water and small solutes, such as glycerol, urea or nitric oxide [NO]) (3). Since their discovery in the early 1990s (4), the functional importance of plasma membrane water channels in mammalian tissues has been extensively studied by analysing the phenotype of

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Correspondence to: Dr Amaia Rodríguez, Metabolic Research Laboratory, University of Navarra, Irunlarrea 1, 31008 Pamplona, Spain. Tel.: +34 948 42 56 00 (ext. 6544), Fax: +34 948 42 56 52, E-mail: arodmur@unav.es

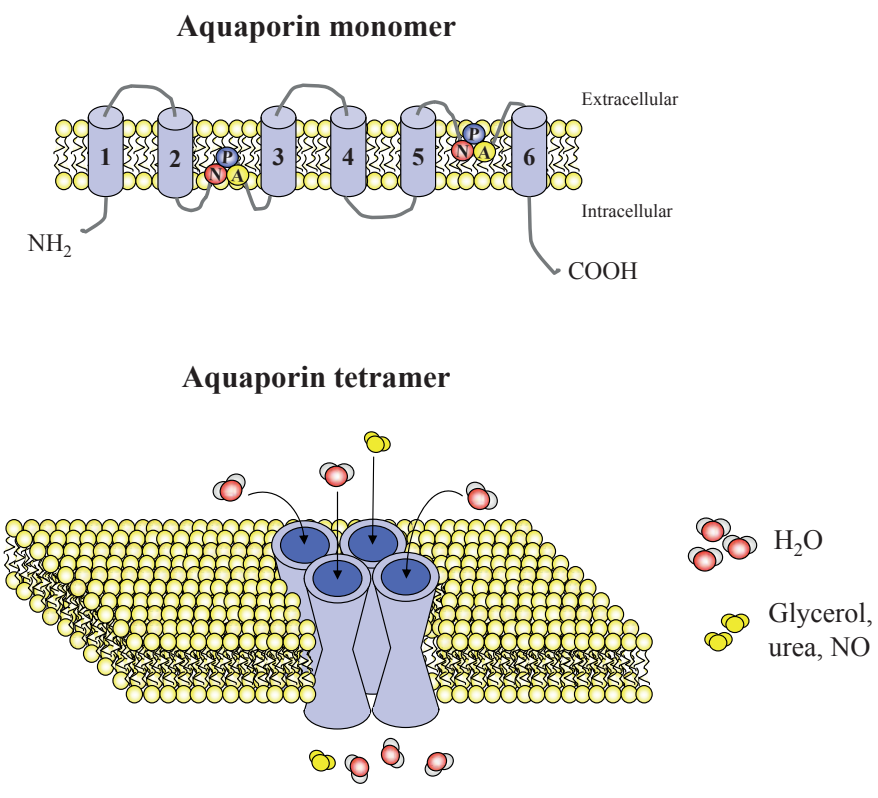

Figure 1. Secondary and tertiary structure of aquaporins. Aquaporins are a family of integral membrane proteins that serve as channels for the movement of water and other small solutes across the lipid bilayer. (A) The polypeptide chain of aquaporin is composed of six transmembrane α-helices and two reentrant loops with asparagine-proline-alanine (NPA) motifs; the water channels AQP11 and AQP12 have a unique asparagine-proline-cystein (NPC) motif. (B) In cellular membranes, aquaporins assemble as a tetramer, with each monomer forming a functionally independent pore that allows the selective passage of water and/or other small solutes, such as glycerol, urea or nitric oxide (NO).

Table 1. Phenotype of aquaporin deficiency in mice and humans AQP, aquaporin.

Aquaporin	*Aqp*-knockout mice	*AQP*-deficient humans
AQP0	Cataracts (49)	Congenital cataracts (50)
AQP1	Polydipsia, defective proximal fluid reabsorption, impaired angiogenesis and vasodilation (55; 56; 60)	Loss of Colton blood group, decreased urine-concentrating mechanism after water deprivation (54; 60)
AQP2	Severe urinary concentrating defect (105)	Nephrogenic diabetes insipidus (65)
AQP3	Defective skin hydration, nephrogenic diabetes insipidus (15; 18; 19)	Antibodies against GIL blood group (14)
AQP4	Reduced brain swelling and improved outcome in models of brain oedema, mild urine-concentrating defect (106-108)	Not described
AQP5	Impaired saliva and sweat secretion, hiperresponsive bronchoconstriction (72; 77; 109)	Not described
AQP6	Not described	Not described
AQP7	Adult-onset obesity, increased insulin production and insulin resistance (29-31; 35)	Impaired increase of serum glycerol during exercise (36)
AQP8	Larger testes (87)	Not described
AQP9	Defective glycerol metabolism (110)	Not described
AQP10	Murine *Aqp10* gene is a pseudogene (46)	Not described
AQP11	Vacuolisation and cyst formation of the proximal tubule leading to polycystic kidney development (100; 101).	Not described
AQP12	Increased susceptibility to caerulein-induced acute pancreatitis (103)	Not described

transgenic knockout mice lacking different aquaporins (Table 1). The present review focuses on advances in our knowledge of the physiological and pathophysiological roles of aquaporins in rodents and humans.

Aquaglyceroporins

Aquaglyceroporins (AQP3, AQP7, AQP9 and AQP10) encompass a subfamily of aquaporins permeable not only to water, but also to small solutes, like glycerol (1,5,6) (Fig. 2). Glycerol represents an important metabolite for the control of fat accumulation, as the carbon backbone of triglycerides (TG), and for glucose homeostasis, given that glycerol constitutes the major substrate for hepatic gluconeogenesis during fasting (7; 8). Circulating glycerol results from lipolysis, diet-derived glycerol or glycerol reabsorbed in proximal tubules (6). During fasting hepatic glucose output embodies the main source of plasma glucose, and plasma glycerol becomes the major substrate for hepatic gluconeogenesis (9,10). In addition, glycerol constitutes a key metabolite for lipid accumulation since it is the carbon backbone of triglycerides (TG). Thus, the regulation of glycerol transport by aquaglyceroporins contributes to the control of fat accumulation and glucose homeostasis, among other biological functions (8,11) (Fig. 3).

AQP3: urine concentration and skin hydration

AQP3 was initially cloned from rat kidney (originally named, GLIP, glycerol intrinsic protein, based on its glycerol transport function) by two independent research groups (12; 13). Although extremely rare, there are cases of homozygous mutations in *AQP3* gene in humans (14). These *AQP3*-deficient patients de-

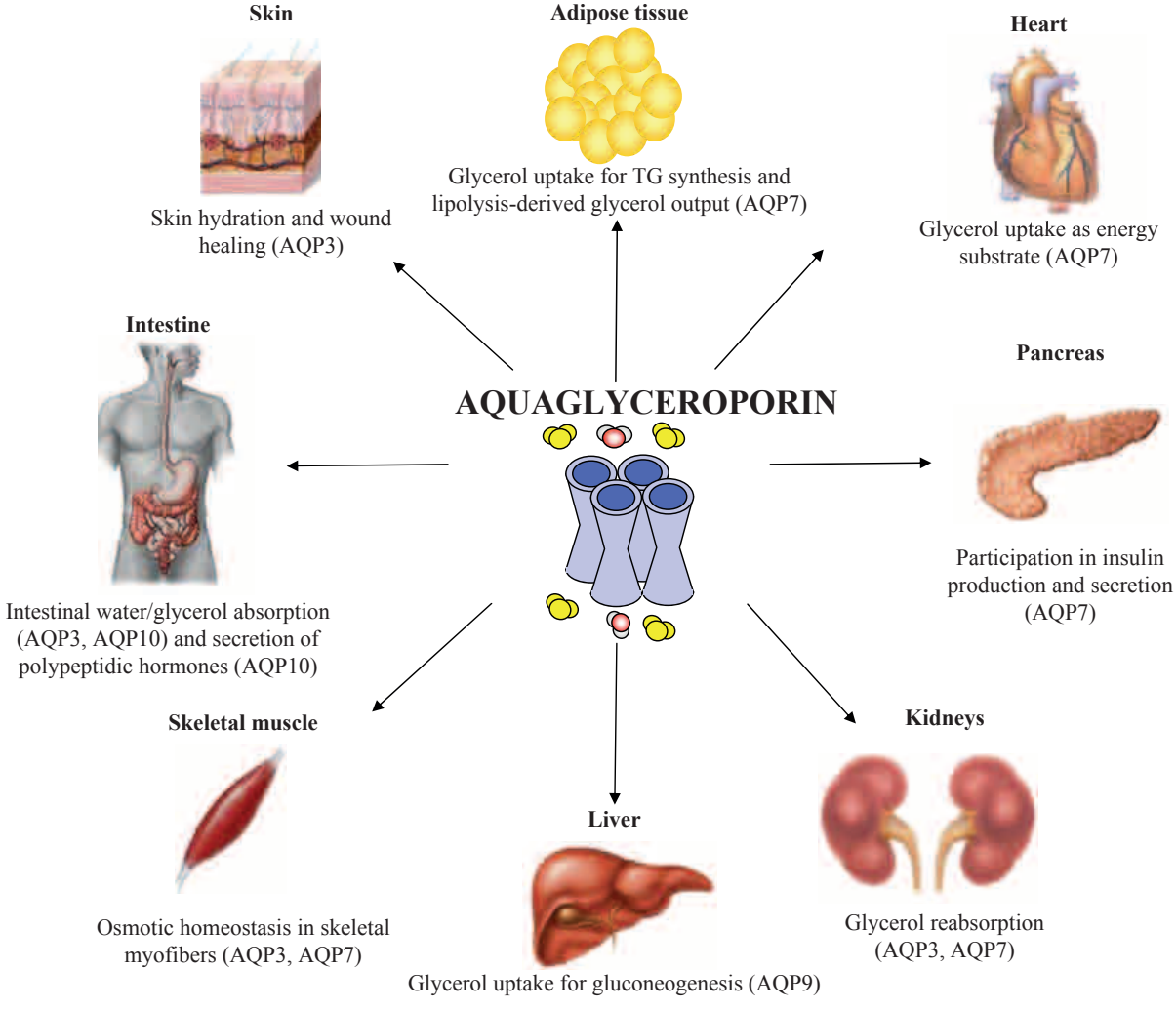

Figure 2. Role of aquaglyceroporins in the human body. The main aquaglyceroporins (AQP) are schematically represented indicating their location and function. TG, triglycerides.

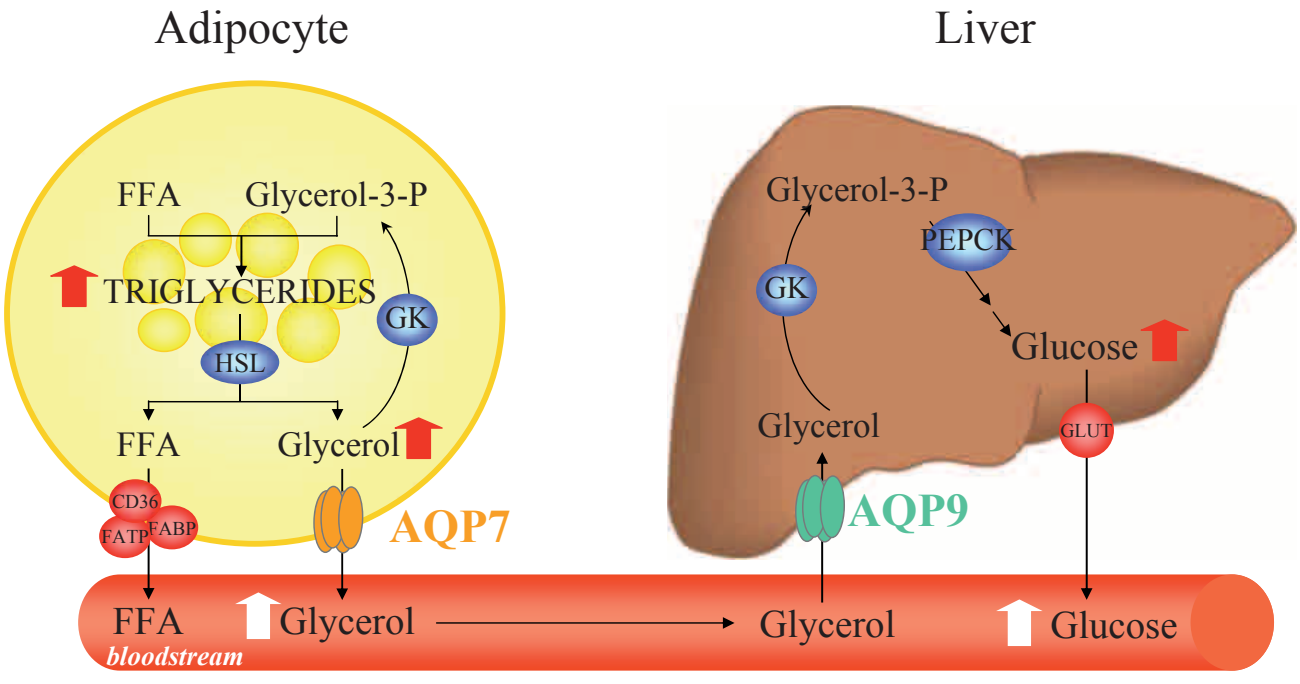

Figure 3. Coordinated regulation of adipose AQP7 and hepatic AQP9 in a fasting state. Under circumstances of negative energy balance, triglycerides are hydrolysed by the hormone sensitive lipase (HSL) to glycerol and free fatty acids (FFA) and released to the bloodstream. Glycerol is secreted from the adipose tissue through AQP7, a glycerol channel mainly expressed in adipocytes during late adipogenesis. The hepatic-specific AQP9 enables the direct flow of glycerol from the portal vein into the liver. In hepatocytes, glycerol is converted to glycerol-3-phosphate by the enzymatic activity of glycerol kinase (GK) for *de novo* synthesis of glucose. Taken together, the glycerol cascade from adipose tissue to liver is maintained by coordinated regulation of AQP7 and AQP9. PEPCK, phosphoenolpyruvate carboxykinase.

veloped antibodies against a new red-blood cell group protein after blood transfusion or pregnancy, but the clinical significance of this biological function of AQP3 has not been determined. Until now, most of the studies have been focused on the role of AQP3 in renal water absorption and skin hydration (15,16). In the kidney, AQP3 is expressed in the apical and basolateral membranes of the proximal tubules (12,13). This aquaglyceroporin plays a key role in the urinary-concentrating mechanism, since *Aqp3* deletion in transgenic mice is associated with nephrogenic diabetes insipidus development (15). Interestingly, it has been recently reported that a mouse model of type 1 diabetes mellitus shows a decrease in AQP3 in renal inner medulla that serves as a compensatory mechanism to alleviate dehydration in diabetes mellitus (17). In the skin, AQP3 is expressed in keratinocytes of the most superficial layer, the stratum corneum (16). Mice lacking *Aqp3* gene exhibit a reduced skin hydration, and elasticity, together with an impaired reformation of stratum corneum and delayed wound healing (18,19). Interestingly, glycerol administration, by topical or systemic routes, has been able to correct each of the phenotype abnormalities in the skin of *Aqp3*-deficient mice (20). These findings suggest that AQP3 plays an important role in skin hydration contributing to glycerol transport across the keratinocytes. This fact might provide a scientific rationale for the long-standing practice of including glycerol in cosmetic and skin medicinal preparations.

AQP7: control of fat accumulation

The human *AQP7* gene, mapped to chromosome 9p13, was cloned from adipose tissue in 1997 (originally named AQPap) (21,22). AQP7 was initially described as an adipose-specific glycerol channel, but this pore is also expressed in kidneys, testes, ovaries, heart, gastrointestinal tract, and skeletal muscle (23-28). The glycerol channel AQP7 plays a pivotal role in adipose tissue enlargement and function as well as glucose homeostasis, since mice lacking *Aqp7* gene have been shown to develop adult-onset obesity and type 2 diabetes mellitus (29-31). The main reason for the adipocyte enlargement in *Aqp7*-deficient mice is the progressive hypertrophy of fat cells, characterised by larger

sized lipid droplets (30,31). In circumstances of negative energy balance, such as fasting or exercise, TG stored in adipocytes are hydrolysed to glycerol and fatty acids by the hormone-sensitive lipase (HSL) and released into the bloodstream (5,32). AQP7 facilitates the secretion of glycerol from adipocytes (Fig. 3). Thus, a defective glycerol exit results in intracellular glycerol accumulation. Increased adipocyte glycerol concentrations would then increase TG biosynthesis, resulting in a progressive adipocyte hypertrophy (30).

Insulin represses *AQP7* expression in adipocytes through the negative insulin response elements (IRE) in the promoter regions of this gene (33). Insulin-resistant *db/db* mice states show an increased expression of the fat-specific AQP7 despite their hyperinsulinaemia (34). The increase of this aquaglyceroporin in the setting of insulin resistance may be caused by impaired IRS-1-mediated insulin signalling in adipocytes (33; 34). Moreover, it is remarkable that AQP7 is also expressed in pancreatic b cells while mice lacking the *Aqp7* gene displayed reduced b cell mass and insulin content but elevated blood insulin levels (35). Therefore, AQP7 modulates insulin production and secretion, whereas insulin reduces AQP7 expression. This coordinated regulation appears to be necessary for the maintenance of insulinemia and glucose homeostasis.

Only a single human case of homozygous mutation in the coding region of the *AQP7* gene has been reported up to date (36). This subject was neither obese nor diabetic with the only apparent consequence of this mutation being an impaired glycerol increase in response to exercise. Fat depot-specific differences in the gene expression of *AQP7* in human obesity have been reported with an overexpression of *AQP7* in omental adipose tissue suggesting an increase in overall lipolytic capacity, and a repression of *AQP7* in subcutaneous fat pointing to the promotion of an intracellular glycerol accumulation and a progressive adipocyte hypertrophy (37-39).

AQP9: regulation of hepatic gluconeogenesis

AQP9 was cloned from human peripheral leukocytes, and in the liver, lung and spleen (40). The presence of AQP9 in the liver as well as its negative regulation by insulin opened up a new field of research regarding the role of this aquaglyceroporin in glucose homeostasis (34). During fasting, plasma glycerol is introduced into hepatocytes by the liver-specific aquaglyceroporin AQP9, where it is converted into glycerol-3-phosphate by the enzymatic activity of glycerol kinase for *de novo* synthesis of glucose (9) (Fig. 3). After feeding, plasma concentrations of insulin increase and this hormone inhibits the gene expression of *Aqp7* in white adipose tissue and that of *Aqp9* in liver through the negative insulin response elements (IRE) in the promoter regions of these genes. Interestingly, the insulin-resistant obese *db/db* mice exhibit increased transcript levels of the fat-specific *Aqp7* and the liver-specific *Aqp9*, despite their hyperinsulinaemia. The increase of AQP7 and AQP9 in the setting of insulin resistance may be caused by impaired insulin signalling in adipocytes and hepatocytes (33,34). Taken together, under physiological conditions, insulin-mediated regulation of AQP7 and AQP9 may account for the increase or decrease of glycerol release from fat and gluconeogenesis in liver, in order to regulate the glucose production depending on the nutritional state. However, in the context of insulin resistance, the overexpression of AQP7 and AQP9 leads to an increase of plasma glycerol and hepatic glucose production associated with elevated circulating glycerol concentrations, a condition that further aggravates the prevailing hyperglycaemia. It is remarkable that obese patients with type 2 diabetes show a down-regulation of hepatic *AQP9* transcript levels that may reflect a reduced glycerol influx into hepatocytes to decrease hepatic gluconeogenesis in an attempt to avoid a further elevation of hyperglycaemia (39,41).

AQP10: intestinal water and glycerol absorption

AQP10 is abundantly expressed in human duodenum, jejunum and ileum, contributing to intestinal water absorption (42,43). For years a paracellular pathway between epithelial cells has been proposed for the transport of water in the intestine. Since the identification of aquaporins in the gastrointestinal tract a transcellular pathway has been suggested, whereby water may pass across the absorptive epithelia via AQP10 (42). Two different isoforms of AQP10 have been described to be expressed in human small intestine: AQP10v and AQP10 (44). AQP10v is mainly expressed in the capillary endothelial cells of the small intestinal villi being possibly involved in the transport of water absorbed through the intestinal epithelium into blood. In this sense, it has been shown that AQP10v is down-regulated during acute cholera that may reflect a mechanism to reduce the water permeability of the cell membranes and thus limit the secretory response (45). On the other hand, AQP10 is localised in the cytoplasm of the gastro-entero-pancreatic (GEP) endocrine cells. In the small intestine, GEP cells secret several hormones, such as somatostatin, gastrin, glucagon, or motilin. The cellular and subcellular localisation of AQP10 suggests that this aquaglyceroporin participates in the secretion of polypeptidic hormones from GEP cells. AQP10 exerts an important role in glycerol absorption in the small intestine, due to the fact that Western societies are used to consuming high-fat diets (46). Despite the contribution of AQP10 to intestinal water and glycerol transport in humans, *Aqp10* has not been shown to be expressed in mice (46). The murine *Aqp10* gene has multiple structure defects lead-

ing to the production of non-functional proteins. Nevertheless, other aquaporins, such as AQP1, AQP3, AQP4 and AQP7 might compensate the lack of *Aqp10* in the murine intestine.

Aquaporins

Aquaporins are widely expressed in tissues implicated in high rates of active fluid transport. These water channels play important functions in renal water absorption, lacrimation and aqueous dynamics in the eye, cerebrospinal fluid secretion, and generation of pulmonary secretions, among others (Fig. 4).

AQP0: the optical function

AQP0 is the major intrinsic protein (original name MIP26) of lens fiber cells (47). The lens contains a uniquely high protein concentration and low water content to maintain an elevated refractive index for transparency. Because of its low water permeability (48), AQP0 facilitates water removal from fiber cells and it has been shown that heterozygous loss of this MIP is enough to compromise lens transparency (49). In fact, homozygous mutations in the *AQP0* gene are associated with hereditary cataracts in mice and humans, suggesting that this water channel is required for optimal crystalline lens transparency and homeostasis (49-51). The proposed mechanisms for the onset of congenital cataracts include loss of AQP0-facilitated fiber–fiber adherence and impaired fiber cell dehydration (52).

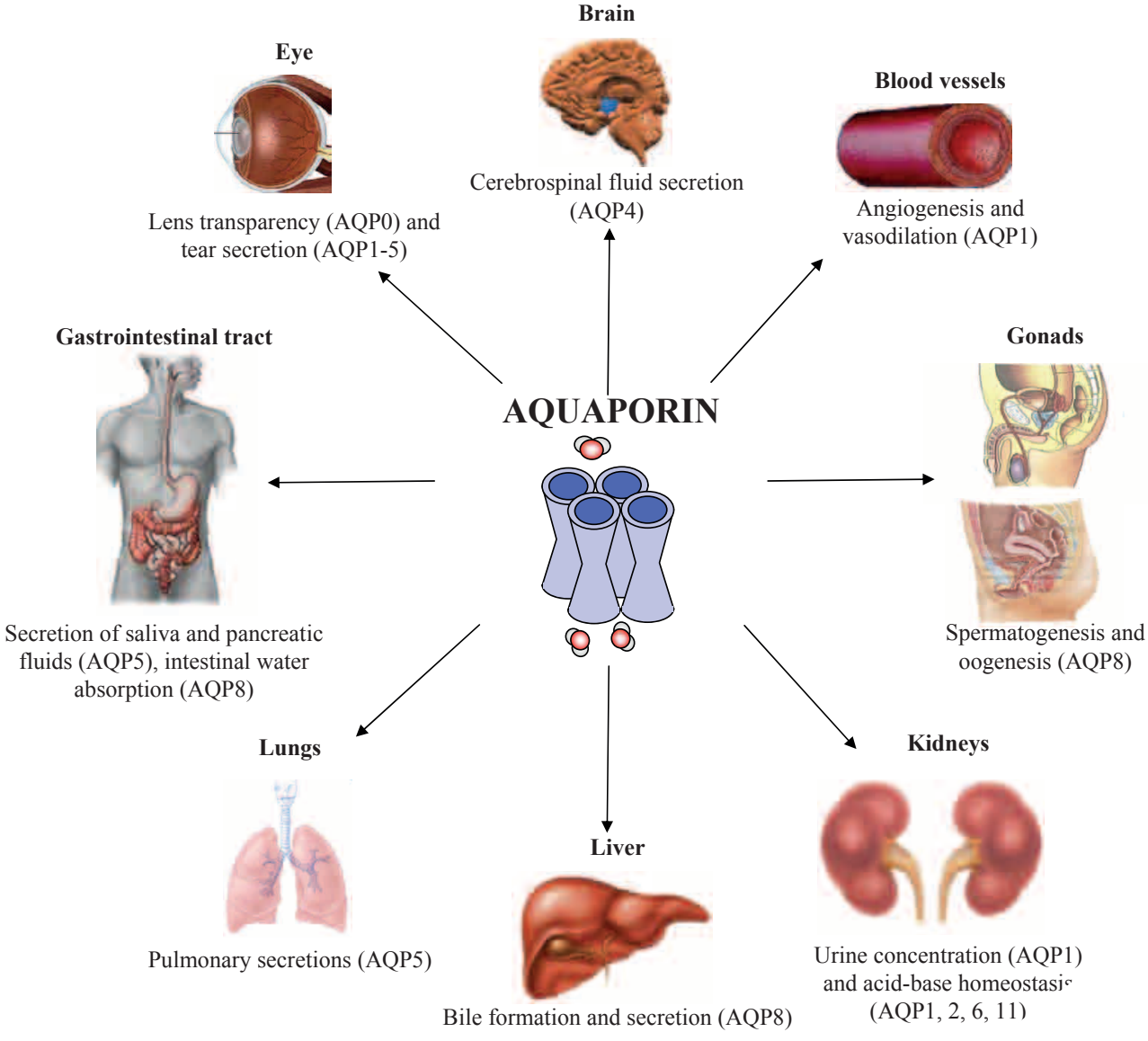

Figure 4. Role of aquaporins in the human body. The main aquaporins (AQP) are schematically represented indicating their location and function.

AQP1: the first aquaporin

The identity of aquaporins remained unknown until the discovery of a protein associated to the red cell Rh blood group antigens, the AQP1 (originally named CHIP28, channel-like integral protein of 28 kDa) (4,53). AQP1 was described as a water-permeable membrane protein of red blood cells, contributing to the Colton blood group antigen, a minor blood group determinant (54). The presence of AQP1 in vascular endothelium and its strong expression in proliferating microvessels of human and rat malignant tumours suggested a possible role of this water channel in angiogenesis (55). AQP1 contributes to endothelial cell migration, which is a key process in angiogenesis. In addition, AQP1 participates in NO-dependent relaxation by facilitating NO efflux from endothelial cells and NO influx into vascular smooth muscle cells (56). The impaired angiogenesis and endothelial-dependent vasodilation in *Aqp1* transgenic knockout mice further confirm these vascular actions of AQP1 (55,56).

The high expression of AQP1 at the apical and basolateral membranes of the proximal tubules suggested an essential role in the renal urine-concentrating mechanism (57,58). Transgenic knockout mice lacking the *Aqp1* gene had increased urine output (polyuria) and decreased urine-concentrating ability (59). After water deprivation for 36 h, *Aqp1*-deficient mice became profoundly dehydrated due to their urine-concentrating defect, which further confirmed that AQP1 is required for the formation of concentrated urine. Humans with a homozygous mutation in the *AQP1* gene were not polyuric, presenting normal glomerular filtration rates, free water clearance, or lithium clearance (indices of proximal tubule function) (60). However, it was observed in a controlled clinical study that *AQP1*-null subjects were unable to concentrate urine after 24 h of thirsting. Thus, *AQP1*-null individuals are at risk for life-threatening clinical problems if they become dehydrated due to renal illness or environmental causes.

AQP2: vasopressin-inducible aquaporin

AQP2 was cloned from the apical membrane of kidney-collecting tubules (originally named WCH-CD, collecting duct water channel protein) (61). Renal water reabsorption and urine concentration is an important mechanism to maintain constant plasma osmolarity of the body fluid compartments. In the kidney, AQP2 trafficking mediates water transport across the apical cell membrane in principal cells of the collecting ducts. The anti-diuretic hormone vasopressin stimulates its receptors on the principal cells in the collecting ducts of nephron, triggering an increase in cAMP that results in the up-regulation of AQP2, rendering the cell permeable to water and, hence, favouring water reabsorption and urine concentration (62-64). In nephrogenic diabetes insipidus, the kidney fails to concentrate urine in response to vasopressin. In this sense, autosomal recessive mutations in the *AQP2* human gene has been shown to cause nephrogenic diabetes insipidus (65). Thus, an abnormal up- or down-regulation of the AQP2 water channels in the principal cells seems to be an important pathophysiological factor in the development of concentrating and diluting defects in progressive renal disease. In this sense, patients with moderately severe chronic kidney disease have a reduced renal concentrating and diluting capacity compared to patients with milder chronic kidney disease as well as healthy control subjects. These phenomena can be attributed, at least partly, to an abnormally decreased response in the vasopressin-cAMP-AQP2 axis (63).

AQP4: brain function

The cloning of AQP4 cDNA from rat brain and lungs (with the original name of the protein being MIWC, mercurial-insensitive water channel) was reported by two independent groups (66,67). AQP4 participates in different physiological processes, including the urinary concentrating mechanism or the resolution of alveolar oedema (67). Nonetheless, AQP4 constitutes the predominant water channel in mammalian brain and most of the studies as regards AQP4 have focused on its role in this organ (3; 68). The brain is composed by two main cell types: neurones, which process and transmit information, and glial cells, which maintain the homeostasis of neurones. AQP4 is mainly expressed in glial cells (66). In the blood-brain barrier, AQP4 is present in the astrocyte projections around blood vessels and, at a lower level, in endothelial cells. AQP4 is further expressed at sites of ependymal cells in contact with the brain-cerebrospinal fluid barrier. This expression pattern indicates that AQP4 is involved in water homeostasis in the brain (69). Phenotype analysis of transgenic mice lacking *Aqp4* gene has provided evidence of other roles of this aquaporin, such as the involvement in brain oedema, in glial cell migration and in neuronal signal transduction (68). In particular, AQP4 facilitates clinically important water movement into and out of the brain in the development and resolution of brain oedema and modulation of AQP4 expression or function is also predicted to modulate glial scar formation, which may be of clinical utility in traumatic injury, tumour and infection. Moreover, recent data suggest an increase in extracellular space volume in AQP4 deficiency and an impaired K^+ reuptake by *AQP4*-null astrocytes, which may be related to functional significant AQP4-K^+ channel interactions (69). It is interesting to note the first case report of a patient with anti-AQP4 antibody who presented with recurrent hypersomnia as the main symptom, symmetrical hypothalamic lesions as well as a reduced orexin (hypocretin) level in the cerebrospinal fluid

(70). Based on the properties of AQP4 in the brain, it has been proposed that regulation of AQP4 might be useful as a novel therapeutic strategy against hydrocephalus, traumatic brain injury, epilepsy and stroke (3; 68).

AQP5: saliva, tears and pulmonary secretions

AQP5 was cloned from rat lacrimal, salivary and respiratory tissues (71). AQP5 is implicated in the generation of saliva and tears. In fact, *Aqp5* deletion in mice is associated with production of low volume hypertonic viscous saliva (72). The presence of AQP5 in the acinar cells of lacrimal and salivary glands opened up the hypothesis that abnormalities in AQP5 expression in these secretor glands may occur in patients with primary Sjögren's syndrome (PSS), an autoimmune disorder that is clinically characterised by dry eyes and mouth (73,74). This hypothesis remains unclear, since other authors discarded a major role of AQP5 in the pathogenesis of PSS because they did not observe changes in the distribution and expression of AQP5 in patients suffering this disease (75).

The importance of AQP5 to human disease may also include disorders in lung and airways (76). AQP5 is expressed in alveolar type I and II cells as well as in the tracheal and bronchial epithelium of mice conferring high osmotic water permeability (71,77). *Aqp5*-null mice present bronchial hyperactivity after cholinergic stimulation, suggesting a physiological role of AQP5 in modulating airway responsiveness and bronchoconstriction (77). Interestingly, some forms of human asthma have been linked to chromosome 12q close to the site where the *AQP5* gene is located. Nevertheless, a role for AQP5 in human asthma has not yet been studied. Moreover, AQP5 has been also implicated in the proliferation and metastasis of lung cancer and its expression is highly increased in human lung adenocarcinomas (78).

AQP6: acid-base homeostasis

AQP6 has been cloned from the rat (WCH3) and human (hKID) kidney (79). In glomeruli, AQP6 is present in the membrane vesicles within the cell bodies of podocytes, suggesting a possible role in glomerular filtration (80). AQP6 also resides in the intracellular vesicles of acid-secreting α-intercalated cells of the collecting duct of the kidney (80,81). In these vesicles AQP6 colocalizes with the H^+-ATPase, a protein that participates in the secretion of acid into the urine. Intercalated cells respond to acid-basic changes by translocating H^+-ATPase from the cytoplasmic vacuoles to the apical plasma membrane, where this ion pump secretes proton from the cells by using ATP supplied by the numerous mitochondria (81). Interestingly, AQP6 permeates anionic ions, especially nitrate, as well as water (82). In this regard, AQP6 expression in collecting ducts increases in response to chronic metabolic alkalosis or increased water intake (83). Thus, AQP6 probably participates in the maintenance of acid-base homeostasis through the regulation of proton excretion by increasing the transport of water through the apical plasma membrane.

AQP8: digestive fluid secretion and reproductive function

Three independent research groups reported the cloning and functional analysis of AQP8 in 1997 (84-86). AQP8 transcript expression has been found in different organs of the digestive system, such as salivary glands, pancreas, liver, gallbladder, small intestine and colon. Several possible functions have been proposed for AQP8, including secretion of saliva and pancreatic fluid, as well as intestinal fluid absorption/secretion (87). The presence of AQP8 in the hepatobiliary system is important for bile formation and secretion with defective expression of hepatocyte AQP8 contributing to bile secretory dysfunction in cholestasis (88; 89). Bile formation is initiated by hepatocytes and is modified by secretory and absorptive processes in the epithelial cells of the intrahepatic ducts and gallbladder. In spite of its intracellular location in hepatocytes, under basal conditions, AQP8 is inserted into the plasma membrane to facilitate the transport of water together with AQP9 in response to hormonal stimuli, such as glucagon (90). The hormone glucagon stimulates hepatocyte bile formation and it induces AQP8 vesicle trafficking to the hepatocyte canalicular domain (91). Thus, glucagon increases the AQP8-mediated osmotic membrane water permeability, facilitating the movement of a process likely to be relevant to glucagon-induced bile secretion. In the gallbladder, AQP8 and AQP1 also contribute to the water absorption and secretion required for bile formation and secretion (92).

The discovery of AQP8 in testes and ovaries has provided information for better understanding central processes that require water movement in the biology of reproduction (24; 84). In the male reproductive tract AQP8 is uniformly expressed in the Sertoli cells, primary spermatocytes and elongated spermatids of the seminiferous tubules (84; 93). This ontogeny and distribution indicate that AQP8 is involved in the secretion of fluid to form the lumen of seminiferous tubules occurring during testes development and the fluid movements during spermatogenesis and sperm concentration and maturation. On the other hand, several physiological processes need water movement in the female reproductive tract. Oocytogenesis or conversion of the oocyte into the mature ovum requires the formation and expansion of the fluid-filled antrum surrounding the cell. The water influx into ovarian antral follicles is mediated by AQP7, 8 and 9 expressed in the granulosa cells (24). Besides its participation in the gametogenesis, AQP8 is also involved in early stages of preg-

nancy (94). The implanting blastocyst expresses both AQP8 and AQP9, probably for fluid/solute transport during the embryo/placental development.

AQP11 and AQP12: the superaquaporins

The completion of the Human Genome project revealed two more aquaporin-like genes, AQP11 and AQP12 (original names AQPX1 and AQPX2) (95). In contrast to conventional aquaporins showing two highly conserved NPA boxes (Fig. 1), AQP11 and AQP12 have a NPA box and a unique asparagine-proline-cystein (NPC) motif (96; 97). AQP11 and AQP12 were grouped as superaquaporins, since they belong to the aquaporin superfamily with very low homology to conventional aquaporins.

AQP11 is highly expressed in rat testis while being moderately expressed in the kidney, liver and brain (98). To gain insight into the physiological role of AQP11, a transgenic mice lacking *Aqp11* gene was produced by Morishita *et al* (99). Although *Aap11* deletion was not lethal, most knockout mice died before weaning. The cause of death was the advanced renal failure due to vacuolisation and cyst formation of the proximal tubule, leading to polycystic kidney development. To a lesser extent, vacuoles were also observed in other organs (the liver and the small intestine). The vacuoles are mostly originated from the endoplasmic reticulum, suggesting that *Aqp11*-knockout mice may have intravesicular defects leading to the accumulation of unprocessed substances inside the vacuoles (100,101). Thus, AQP11 seems to play a relevant role in intravesicular homeostasis, which is essential for an adequate proximal tubular function.

AQP12 is selectively expressed in the pancreas (95). The exocrine pancreas has the ability to secrete daily large amounts of fluid into the duodenum (1-2.5 litres of juice containing digestive enzymes in humans). The intracellular localization of AQP12 in pancreatic acinar cells suggests a potential role of this water channel in the maturation and exocytosis of zymogen granules (102). Nevertheless, the deficiency of *Aqp12* in transgenic mice did not affect the overall pancreatic exocrine function under a normal breeding environment, but *Aqp12*-knockout mice showed a more severe pathology resulting from cholecystokinin-8 analog-induced pancreatitis than wild type mice (103).

Conclusion

The discovery of aquaporins and the analysis of the phenotype of transgenic mice lacking different aquaporins has led to substantial advances in the life and medical sciences (Table 1) (104). The impaired function of aquaporins has been associated with several human diseases, such as congenital cataracts or nephrogenic diabetes insipidus. These studies have provided new insights into the underlying mechanisms of well-known human diseases, indicating that pharmacological modulation of water and/or glycerol transport targeting aquaporins may provide novel opportunities for therapeutic interventions in several human disorders. From a clinical point of view, the possibility of regulating the expression of aquaporins in several tissues offers potentially different therapeutic approaches for a number of diseases. In this context, the design of small-molecule modulators of aquaporin expression/function may have clinical applications in the therapy of congestive heart failure and hypertension (AQP1 and AQP2 inhibitors), cytotoxic and vasogenic types of brain swelling (AQP4 modulators), obesity (AQP7 up-regulators), and tumour angiogenesis (AQP1 inhibitors), among others. Nonetheless, additional data, related to gene expression and protein stability are needed to better establish a firm mechanistic basis for the involvement of aquaporins in the ethiopathogenesis of these metabolic disorders. Undoubtedly, aquaporins have broadened our understanding of the implications of water balance as well as water/glycerol transport to mammalian pathophysiology. Given the versatile functions of aquaporins, additional and unexpected roles of these channels are sure to emerge in the coming years.

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