

## Aquaporins; systemic, functional and therapeutic correlations in health and disease

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### Abstract

Aquaporins are transcellular proteins that majorly consist of a tetrametric hour-glass-like structure. Aquaporins have a definitive function in transpositioning of water and solutes across cell membranes. The current review was planned to provide information regarding structure, expression and functions of aquaporins in the human body, and to generate a comprehensive summary of physiological and pathophysiological correlations related to aquaporins and their therapeutic implications. Absence or mutations of different aquaporins are believed to be linked to clinical diseases, including Sjogren's syndrome, nephrogenic diabetes insipidus, liver dysfunction and obesity. Modern therapeutic techniques are utilising aquaporins in gene therapy for Sjogren's syndrome and cholestasis. The narrative review aims at providing a comprehensive description of localisation, function, abnormal clinical conditions and therapeutic implications of aquaporin proteins.

**Keywords:** Classification, Clinical, Human aquaporins, Localisation, Structure, Water channels.

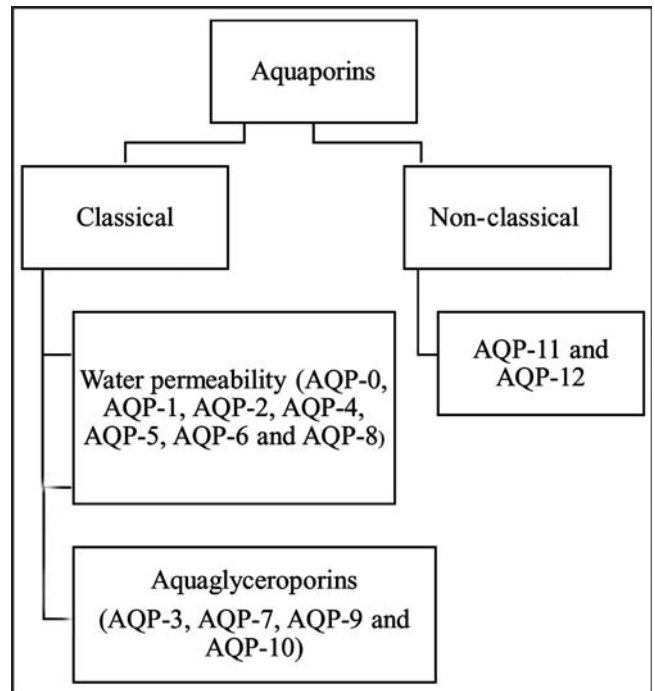
**DOI:** <https://doi.org/10.47391/JPMA.01-063>

### Introduction

Aquaporins (AQPs) are a lineage of transmembrane proteins which assist in transpositioning across the cell membranes, mainly consisting of water and solutes.<sup>1</sup> At least 13 types of AQPs have been identified (AQP-0 to AQP-12). They are divided into classical and non-classical categories. Classical AQPs include the ones that are involved in water permeability; AQP-0, AQP-1, AQP-2, AQP-4, AQP-5, AQP-6 and AQP-8). Classical AQPs also include aquaglyceroporins; AQP-3, AQP-7, AQP-9 and AQP-10. In addition to water, they also assist in the transport of glycerol and some neutral solutes. Non-classical AQPs, also referred to as sub-cellular or supraaquaporins, include AQP-11 and AQP-12, and their function in controlling the water permeability is not well-established and the exact physiological role is yet to be determined<sup>2</sup> (Figure-1).

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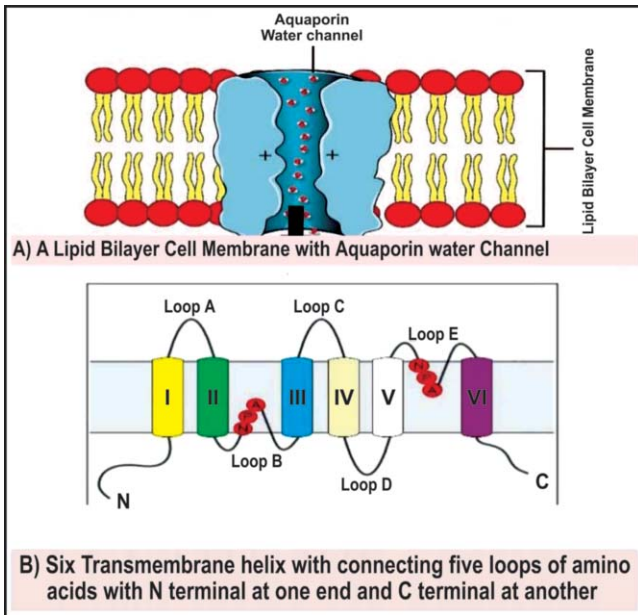


**Figure-1:** Schematic representation of classification of Aquaporins (AQPs).

### Structure of Aquaporins

Aquaporins are small, identically hydrophobic membrane proteins that consist of monomers. Each monomer comprises six transmembrane helix, making AQPs act as a hydrophobic integral membrane protein.<sup>3</sup> The monomers vary in sizes and mammalian AQPs range 26-34KDa.<sup>4</sup> These monomers contain amino acid sequences, making a bilayer membrane that contains five connecting loops from A to E. Loop B and E contains classical asparagine-proline-alanine (NPA) motifs in all AQPs<sup>5</sup> (Figure-2).

The structure of AQP-1 is exclusive due to its specialised permeability features which include three transmembrane domains and B and E loops containing several subunits consisting of trademark repetition of NPA. These subunits form an intrinsic aqueous pore which facilitates transport of water, and forms an hour-glass model that results from the folding of the loops B and E.<sup>6</sup> Studies based on light microscopy and three-dimensional (3D) microscopy show the structure of AQP-1 consists of a lipid bilayer which



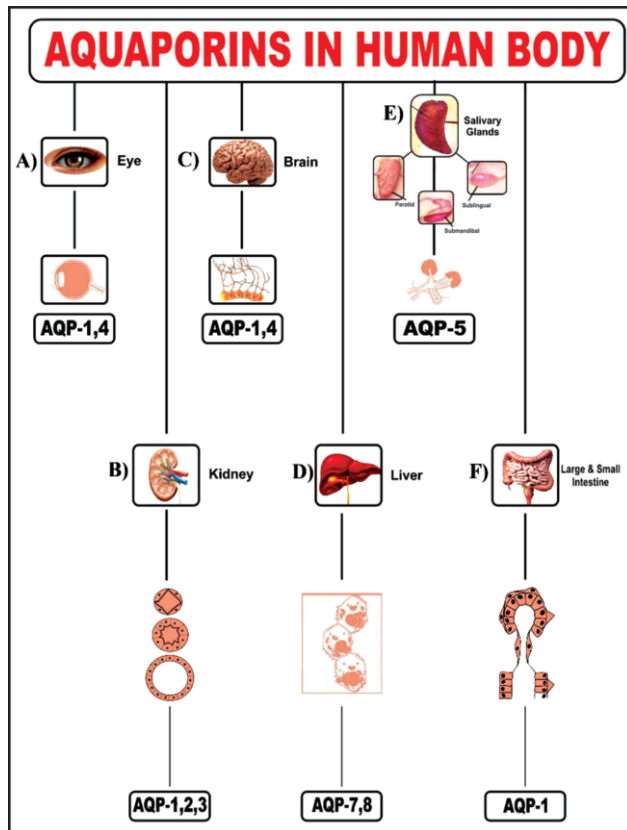
**Figure-2:** The proposed structure of Aquaporins (AQPs). A) A lipid bilayer cell membrane with AQP water channel. B) This is an 'hour-glass' model that consists of six transmembrane helices (I, II, III, IV, V, VI) with connecting five loops of amino acids with N terminal at one end and C terminal at the other. Loop A connects helices I and II, Loop B connects helices II and III and has asparagine-proline-alanine motif (NPA) with it. Loop C connects helices III and IV and Loop D connects IV and V. Loop E connects V and VI and has NPA motif. The image is modified from Bai et al, 1996.<sup>38</sup>

exists as a 'tetramer' in which each subunit consists of its own pore.<sup>7</sup> AQP-1 and AQP-2 consist of four exons and have an identical exon-intron boundary.<sup>8</sup> The subunit of AQP-2 relates to the structure of AQP-1 as a tetrameric structure and is persistent as an hour-glass model with minor alterations of crystal sheets consisting of densities of coats sandwiched between isolated layers at the region of N and C terminals.<sup>9</sup> The structure of AQP-3 also resembles the structure of other AQPs as it also consists of six transmembrane domains.<sup>10</sup> AQP-3 consists of six exons in comparison to four exons of AQP-1 and AQP-2, and, having a different sequence of exon-intron boundary, suggests that AQP-3 has a different developmental branch compared to other AQPs. Similar to AQP-1, AQP-4 also consists of a tetra-metric structure with six transmembrane helices.<sup>8</sup> The structure of AQP-5 is similar to other AQPs that consists of tetramers and each tetramer consists of six transmembrane domains with B and E loops. AQP-5 differs from other AQPs as it has slight C terminal modification and its crystals lack typical four-fold symmetry because the crystals of AQP-5 imitates the identical triad of plant APQs such as spinach AQP phosphatidylinositol-4,5-bisphosphate (SoPIP<sub>2</sub>;1) water channel, which is a major integral protein of spinach AQPs.<sup>11,12</sup>

## Localisation and function of AQPs

In mammals, AQP-1 is localised in the proximal tubules and thin loop of Henle's apical and basolateral membrane where it acts as a pathway for water from tubular lumen to interstitium.<sup>13</sup> In humans, AQP-1 is also involved in secretory regulation of cerebrospinal fluid (CSF). In the eye, it regulates the secretion of aqueous humour which is located in the anterior chamber of the eye. AQP-1 also regulates the secretory regulation of bile produced by the liver and in lungs, and it also regulates bronchial circulation.<sup>14</sup> AQP-1 is found to be localised on the endothelial and myoepithelial cells of salivary glands of humans.<sup>2</sup> In humans, AQP-2 functions as a secretory regulator of water channels in the renal collecting tubules, acting on vasopressin-water channels. AQP-2 is systemised by the mechanism of exocytosis which is the receptor-moderated action of adenylyl cyclase-protein kinase A phosphorylation. AQP-2 in the apical membrane provides an intracellular pathway for water to move from the lumen of the renal collecting ducts into the interstitium.<sup>3,15</sup>

In mammals, AQP-3 is localised at the basolateral membranes of the renal collecting ducts, colon, small intestine, kidney, liver, lungs and plasma membrane of skeletal muscles.<sup>16</sup> In humans, the salivary glands are categorised as exo-merocrine glands that consist of several basolateral membranes which contain groups of cells called acinar cells.<sup>6</sup> These acinar cells are further subdivided into serous or mucus and AQP-3 is often localised in membrane of these cells. During the maturation stage of formation of enamel, the process of modulation takes place and ameloblasts modulate in possessing either a ruffled border or a smooth border. It is assumed that AQP-3 plays a functional role in assisting modulation of ameloblast during the maturation stage of enamel formation.<sup>17</sup> AQP-3 has a critical role in sperm osmoregulation because AQP-3 is present on the sperm's tail, and helps in volume-regulation by prohibiting cellular swelling in the female reproductive tract and maintaining the structural integrity of sperms.<sup>18</sup> In humans, AQP-4 is also an important water-channel protein mostly located in the brain and is a site of action of many drugs that are used as therapeutic modalities for cerebral oedema, bipolar disorders and medial temporal lobe epilepsy. These anti-epileptic drugs include diazepam and phenobarbitals.<sup>19</sup> AQP-4 exists in glial cells as a full-length protein and is localised in glial lamellae surrounding vasopressin-secretory neurons. In addition, AQP-4 is found in the sarcolemma of fast-twitch fibres in skeletal muscle. The hypothalamus plays important physiological roles that include osmo-regulation, thermo-regulation and glucose-osmo



**Figure-3:** Schematic presentation of localisation of different types of aquaporins (AQPs) in human tissues and organ. A) AQP-1 and AQP-4 are found in anterior chamber of the eye and epithelial cells of cornea of eye. B) AQP-1, AQP-2 and AQP-3 are found in apical membrane of renal collecting tubules of kidney. C) AQP-1 and AQP-2 are located in glial cells of hypothalamus. D) AQP-7 and AQP-8 are found in the plasma membrane of adipocytes and mitochondrial membrane in hepatocytes. E) AQP-5 is found in the basement membrane of acinar cells of salivary glands. F) AQP-1 is found in the basolateral membrane of large and small intestine. The figure has been conceptualised in line with Mescher et al, 2018, and Drake et al, 2019.<sup>6,39</sup>

regulation. AQP-4 is localised in different regions of hypothalamus, including glial lamella where it is believed that AQP-4 may play a physiological functioning role in osmo-regulation, thermoregulation and glucose-osmo regulation<sup>20</sup> (Figure-3).

AQP-5 is extensively distributed in different regions of the human body, including submucosal, sweat, salivary and lacrimal glands, from digestive to renal, respiration and reproductive systems.<sup>21</sup> In the human eye, AQP-5 is present in plasma membrane of epithelium of lens fibre cells, and plasma membrane of corneal epithelium. It is also localised in the apical and basolateral membranes of acinar cells of lacrimal and salivary glands.<sup>21</sup> Physiologically, it is believed that AQP-5 helps in the production of primary saliva and tear formation and their secretory regulation.<sup>22</sup> AQP-5 is also expressed in the

mammary glands, and it is localised in apical membrane of ductal cells in the mammary glands where it is expressed in the apical membrane of ductal cells. In human lungs, AQP-5 is localised in submucosal glands, epithelial cell membrane, and different types of pneumocytes. In the digestive system, AQP-5 is localised in different areas which consists of apical membranes of the pancreas, as well as intercalated and interlobular ductal cells. In integumentary system, AQP-5 is expressed in the sweat glands, keratinocytes and a glandular layer of skin. It is also understood that AQP-5 is present in secretory membranes of sweat glands. AQP-5 is also expressed in renal cortex, in the apical membrane of Type-B intercalated cells in the renal collecting duct. In the female reproductive system, AQP-5 is expressed in the cytoplasm of vaginal epithelial cells, in the basolateral membrane of endometrial glandular epithelial cells and in the plasma membrane of uterus smooth muscle cells.<sup>21</sup>

In mammals, AQP-7 is expressed in the secretory membrane of adipocytes and it functions as an important protein in glycerol transport in adipocytes.<sup>23</sup> AQP-8 is a cross-functional protein channel which is expressed in the inner mitochondrial membrane in hepatocytes. It not only acts as a water channel, but also works in facilitating endorsement of ammonia in hepatocytes, and detoxifies it by converting into urea. It also releases hydrogen peroxide, which in humans reduces cholesterol loading and increases its depletion<sup>24</sup> (Table-1).

### Clinical pathophysiology of AQPs

Lack of AQP proteins in humans suggests several conditions with pathological importance and signifies the importance of AQP-1. The Colton blood (CO) group is a rare protein-based blood group which consists of antigens such as colton allele a (COa), and Colton allele b (COb). In response to these aforementioned antigens the CO group has immunoglobulin G (IgG) based antibodies such as anti-COa and anti-Cob.<sup>25</sup> Polymorphism of AQP-1 makes it impossible for CO individuals to receive a blood transfusion due to its mutation which can lead to transfusion reaction and haemolytic anaemia. AQP-1 mutations also cause defects in urine concentration and disturbance of water movement between vascular space and interstitium. This leads to failure to detect subacute or chronic fluid overload.<sup>26</sup> AQP-2 may be involved in some of the aetiological causes of nephrogenic diabetes insipidus (NDI), and AQP-2 can also act as a potential biomarker for efficacy of drugs, such as tolvaptan-containing drugs that are given in decompensated heart failure complicated by diabetic-nephrotic syndrome.<sup>27</sup> Deficiency of AQP-3 is linked with a type of NDI, and causes impaired wound healing in epidermis of human

**Table-1:** Types of aquaporins (AQPs), localisation and functions.

Aquaporin	Localization	Function	Reference
AQP-1	In human renal system it is expressed in the secretory membranes of thin loop of henle, collecting tubules and anterior chamber of eye, blood cells i,e RBCs and epithelium of lungs	Pathway for water, secretory regulation of cerebrospinal fluid (CSF), aqueous humour and bile.	Nielsen and Agre, 1995, Pallone et al, 1997, Delporte and steinfeld, 2006
AQP-2	Apical membrane of renal collecting ducts	Act as an intracellular pathway for water movement	Agre et al, 2002, Christensen et al, 2000
AQP-3	Basolateral membranes of collecting ducts and colon, skin, small intestine, skeletal muscles, lungs, eye, tail of sperms and acinar cells of salivary glands	Stimulates modulation between smooth and ruffed-ended ameloblasts, maintains structural integrity of sperms	Wang et al, 2003, Chen and Duan, 2011
AQP-4	Glial cells of brain, sarcolemma of fast-twitch muscles, kidney, glial cells of hypothalamus and pancreas of stomach	Water balance in brain, regulation of potassium uptake, astrocyte communications, Site of action of drugs in brain (including anti-epileptics, sodium-potassium-chloride cotranportaper antagonists) osmo and thermo regulation, glucose sensing	Verkman and Mitra, 2000, Hiroaki et al, 2006, Lacovetta et al., 2012
AQP-5	Acinar cells of the ductal membrane of salivary and lacrimal glands, epithelium of lens and cornea, secretory membranes of submucosal, sweat, intercalated and intraductal cells of pancreas, apical membrane of keratinocytes	Primary saliva formation, tears formation	Delporte and Steinfeld, 2006
AQP-7	Plasma membrane of adipocytes	Glycerol transport in adipocytes	Hibuse et al, 2005
AQP-8	Inner mitochondrial membrane in hepatocytes	Detoxification of ammonia to urea in hepatocytes	Danielli et al,2017

skin.<sup>28,29</sup> AQP-4 deficiency leads to disruptions in defects in water potassium ions homeostasis which may contribute to medial temporal lobe epilepsy.<sup>30</sup> AQP deficiency or defects play a role in the pathophysiology of brain oedema, seizure activities, hepatoencephaly and brain tumours.<sup>31</sup>

Mutation of AQP-5 and abnormal localisation has been reported to be related to Sjogren's syndrome (SS) which is characterized by conditions such as dry mouth and dry eyes. In SS, AQP-5 is reported to be present in both acinar and basolateral membrane when compared to non-SS individuals.<sup>32</sup> Defects in AQP-5 transpositioning in salivary glands include parotid gland. Knockout mice revealed a 60% decrease of saliva production when compared to

healthy mice.<sup>33,34</sup> Deficiency of AQP-7 contributes to one of the aetiological mechanisms causing obesity that is mediated through stimulation of an adipose glycerol kinase pathway and it also causes severe resistance to insulin, which, in return, activates the excessive formation of triglycerides (TGs) which also leads to obesity.<sup>35</sup> Malfunctioning of AQP-8 is directly related to increased incidence of obesity<sup>24</sup> (Table-2).

### Therapeutic correlations of AQPs

AQPs play an important physiological role, but may also be useful for modern therapeutic techniques in the light of gene therapy for the treatment of SS and cholestasis.<sup>4,36</sup> SS is an autoimmune disease characterised by dryness of the eyes and mouth and hyposalivation. It

**Table-2:** Clinical implications of different types of aquaporins (AQPs) in human tissues and organs.

Aquaporin	Clinical implication	Reference
AQP-1	Auto-antibodies and defects in urine concentration	Nielsen and Agre, 1995, Smith and Agre, 1991, Zelinski et al, 1990
AQP-2	May cause Nephrogenic-Diabetes Insipidus	Tanaka et al, 2016
AQP-3	May cause Nephrogenic-Diabetes Insipidus	Ma et al, 2000, Sasaki, 2012
AQP-4	One of the etiological factor of Mesial temporal lobe epilepsy, part of pathophysiology of brain edema, hepatoencephalopathy, seizures and brain tumors	Eid et al, 2005, Lacovetta et al., 2012
AQP-5	Xerostomia, Xerophthalmia	Ma et al, 1999
AQP-7	One of the contributing factor for obesity	Hibuse et al, 2005
AQP-8	One of the contributing factor for obesity	Danielli et al,2017

has widespread multiple aetiological factors, one of which is glandular inflammation and degeneration.<sup>37</sup> The treatment regime in the present era for SS is symptomatic relief as no curative treatment exists. However, in experimental animal models, gene therapy is being studied. AQP-1 gene therapy restores salivary and lacrimal fluid movement and decreases glandular inflammation in murine model of SS.<sup>5</sup> AQP-8 plays an important role in bile formation and down-regulation of AQP-8 by oestrogen induces failure or decrease in bile secretion which can cause liver cholestatic diseases that can progress to liver cirrhosis and even liver failure in extreme chronic cholestasis. Gene therapy of AQP-1 to oestrogen-induced cholestatic animal model showed increase water permeability and increased bile secretion, thereby improving the bile secretory dysfunction.<sup>36</sup>

## Conclusion

AQPs have prime importance in maintaining essential bodily functions, from producing saliva and tears to breaking-down of fats, transport and absorption of ions across kidneys, osmo-regulation and thermo-regulation, ammonia detoxification and cholesterol depletion. Deficiency of AQPs is linked to many diseases, including diabetes, SS and obesity. Future studies involving investigations based on linkage of aquaporins with systemic diseases may help in developing strategies that may reduce or completely eradicate aetiological factors and dysfunctions of systemic diseases, like xerostomia, xerophthalmia and obesity.

**Disclaimer:** None.

**Conflict of Interest:** None.

**Source of Funding:** The M.Phil research of Talal Ahmed are fully funded by the Univeristy of Health Sciences, Lahore, Pakistan.

## References

1. Delporte C, Bryla A, Perret J. Aquaporins in salivary glands: from basic research to clinical applications. *Int J Mol Sci.* 2016; 17:166.
2. D'Agostino C, Elkashty OA, Chivasso C, Perret J, Tran SD, Delporte C. Insight into Salivary Gland Aquaporins. *Cells.* 2020; 9:1547.
3. Li C, Wang W. Molecular biology of aquaporins. *Adv Exp Med Biol.* 2017; 969:1-34.
4. Lai Z, Yin H, Cabrera-Pérez J, Guimaro MC, Afione S, Michael DG, et al. Aquaporin gene therapy corrects Sjögren's syndrome phenotype in mice. *Proc Natl Acad Sci USA.* 2016; 113:5694-9.
5. Lai Z, Yin H, Cabrera-Perez J, Guimaro MC, Afione S, Michael DG, et al. Aquaporin gene therapy corrects Sjogren's syndrome phenotype in mice. *Proc Natl Acad Sci USA.* 2016; 113:5694-9.
6. Mescher AL. Junqueira's basic histology: Text and Atlas: McGraw-Hill Education, 2018.
7. Werber JR, Osuji CO, Elimelech M. Materials for next-generation desalination and water purification membranes. *Nat Rev Mater.* 2016; 1:1-15.
8. Li S, Li C, Wang W. Molecular aspects of aquaporins. *Vitam Horm.* 2020; 113:129-81.
9. Sasaki S. Aquaporin 2: from its discovery to molecular structure and medical implications. *Mol Aspects Med.* 2012; 33:535-46.
10. Laforenza U, Bottino C, Gastaldi G. Mammalian aquaglyceroporin function in metabolism. *Biochim Biophys Acta.* 2016; 1858:1-11.
11. Roche JV, Horsefield ST. Aquaporin protein-protein interactions. *Int J Mol Sci.* 2017; 18:2255.
12. Maurel C. Plant aquaporins: novel functions and regulation properties. *FEBS letters.* 2007; 581:2227-36.
13. Li Y, Wang W, Jiang T, Yang B. Aquaporins in urinary system. *Adv Exp Med Biol.* 2017; 969:131-48.
14. Maltaner RE, Schiappacasse A, Chamorro ME, Nesse AB, Vittori DC. Aquaporin-1 plays a key role in erythropoietin-induced endothelial cell migration. *Biochim Biophys Acta Mol Cell Res.* 2020; 1867:118569.
15. Milano S, Carminosino M, Gerbino A, Svelto M, Procino G. Hereditary nephrogenic diabetes insipidus: pathophysiology and possible treatment. An update. *Int J Mol Sci.* 2017; 18:2385.
16. da Silva IV, Rodrigues JS, Rebelo I, Miranda JP, Soveral G. Revisiting the metabolic syndrome: The emerging role of aquaglyceroporins. *Cell Mol Life Sci.* 2018; 75:1973-88.
17. Wang W, Hart P, Piesco N, Lu X, Gorry M, Hart T. Aquaporin expression in developing human teeth and selected orofacial tissues. *Calcif Tissue Int.* 2003; 72:222-7.
18. Cerdà J, Chauvigné F, Finn RN. The physiological role and regulation of aquaporins in teleost germ cells. *Adv Exp Med Biol.* 2017; 969:149-71.
19. Madeira A, Moura TF, Soveral G. Detecting aquaporin function and regulation. *Front Chem.* 2016; 4:3.
20. Moe SEE. Basic mechanisms of RNA interference and the brain water channel Aquaporin-4. 2017; 4:12-23.
21. Direito I, Madeira A, Brito MA, Soveral G. Aquaporin-5: from structure to function and dysfunction in cancer. *Cell Mol Life Sci.* 2016; 73:1623-40.
22. Krueger GF, de Oliveira MC, Gassen HT, Sganzerla JT, Simon D, Grivicich I, et al. Evaluation of Aquaporins 1 and 5 Expression in Rat Parotid Glands After Volumetric Modulated Arc Radiotherapy and Use of Low-Level Laser Therapy at Different Times. *J Lasers Med Sci.* 2020; 11:262-7.
23. da Silva IV, Soveral G. Aquaporins in obesity. *Adv Exp Med Biol.* 2017; 969:227-38.
24. Danielli M, Capiglioni AM, Marrone J, Calamita G, Marinelli RA. Cholesterol can modulate mitochondrial aquaporin-8 expression in human hepatic cells. *IUBMB life.* 2017; 69:341-6.
25. Sutton G, Debono J, Borg J. Determining the frequency of colton blood group antigens Coa and Cob in the maltese population. *Malta J Health Sci.* 2019.
26. Huret JL. AQP1 (aquaporin 1 (Colton blood group)). *Atlas Genet Cytogenet Oncol Haematol.* 2020.
27. Tanaka A, Nakamura T, Sato E, Node K. Aquaporin-2 is a potential biomarker for tolvaptan efficacy in decompensated heart failure complicated by diabetic nephrotic syndrome. *Int J Cardiol.* 2016; 210:1-3.
28. He J, Yang B. Aquaporins in renal diseases. *Int J Mol Sci.* 2019; 20:366.
29. Bollag WB, Aitkens L, White J, Hyndman KA. Aquaporin-3 in the epidermis: more than skin deep. *Am J Physiol Cell Physiol.* 2020; 318:C1144-C53.
30. Vandebroek A, Yasui M. Regulation of AQP4 in the central nervous system. *Int J Mol Sci.* 2020; 21:1603.
31. Tang G, Yang GY. Aquaporin-4: A potential therapeutic target for cerebral edema. *Int J Mol Sci.* 2016; 17:1413.
32. Limaye A, Hall B, Zhang L, Cho A, Prochazkova M, Zheng C, et al. Targeted TNF- $\alpha$  overexpression drives salivary gland

- inflammation. *J Dent Res.* 2019; 98:713-9.
33. Ishikawa Y, Inoue N, Zhenfang Y, Nakae Y. Molecular mechanisms and drug development in aquaporin water channel diseases: the translocation of aquaporin-5 from lipid rafts to the apical plasma membranes of parotid glands of normal rats and the impairment of it in diabetic or aged rats. *J Pharmacol Sci.* 2004; 96:271-5.
  34. Ma T, Song Y, Yang B, Gillespie A, Carlson EJ, Epstein CJ, et al. Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. *Proc Natl Acad Sci USA.* 2000; 97:4386-91.
  35. Hibuse T, Maeda N, Funahashi T, Yamamoto K, Nagasawa A, Mizunoya W, et al. Aquaporin 7 deficiency is associated with development of obesity through activation of adipose glycerol kinase. *Proc Natl Acad Sci USA.* 2005; 102:10993-8.
  36. Marrone J, Lehmann G, Soria LR, Pellegrino JM, Molinas S, Marinelli RA. Adenoviral transfer of human aquaporin-1 gene to rat liver improves bile flow in estrogen-induced cholestasis. *Gene Ther.* 2014; 21:1058.
  37. Alam J, Choi YS, Koh JH, Kwok SK, Park SH, Song YW, et al. Detection of Autoantibodies against Aquaporin-1 in the Sera of Patients with Primary Sjögren's Syndrome. *Immune Netw.* 2017; 17:103-9.
  38. Bai L, Fushimi K, Sasaki S, Marumo F. Structure of aquaporin-2 vasopressin water channel. *J Biol Chem.* 1996; 271:5171-6.
  39. Drake R, Vogl AW, Mitchell AW. *Gray's Anatomy for Students Flash Cards E-Book.* USA: Elsevier, 2019; pp-785.
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