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KATARZYNA MICHALEK

EWA CZERNIAWSKA-PIATKOWSKA

MARTA GRABOWSKA

MARIA LASZCZYNSKA

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Immunohistochemical identification of aquaporin 2 in the kidneys of wild boars (*Sus scrofa*)

Katarzyna MICHAŁEK[1,*], Ewa CZERNIAWSKA-PIĄTKOWSKA[2], Marta GRABOWSKA[3], Maria LASZCZYŃSKA[3]

[1]Department of Physiology, Cytobiology and Proteomics, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology in Szczecin, Poland

[2]Department of Ruminant Sciences, Faculty of Biotechnology and Animal Husbandry, West Pomeranian University of Technology in Szczecin, Poland

[3]Department of Histology and Developmental Biology, Faculty of Health Sciences, Pomeranian Medical University, Szczecin, Poland

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Abstract: Mammalian aquaporins (AQPs) are a family of at least 13 small, integral membrane proteins expressed in various fluid-transporting epithelial cells. Aquaporin 2 (AQP2) is mainly expressed in the kidneys, where it plays an important role in the reabsorption of water from the renal tubular fluid to concentrate urine. Studies of AQP2 expression in the kidneys have been performed in humans, monkeys, sheep, dogs, rabbits, rats, mice, and cattle. We analyzed the expression of AQP2 in the kidneys of wild boars (*Sus scrofa*). AQP2 was localized in the principal cells of collecting ducts in medullary rays penetrating the renal cortex and in the collecting duct of the renal medulla. The presence of AQP2 was confirmed mainly in the apical plasma membrane but also in the intracellular components and basolateral membrane of the principal cells of the collecting ducts. Due to the key role of AQP2 in the regulation of water balance in mammals and the lack of studies in the literature concerning analysis of this protein in wild boars, this work completes the present state of knowledge by providing new information on immunolocalization and immunoexpression of aquaporin 2 in *Sus scrofa* kidneys.

Key words: Aquaporin 2, wild boars, immunohistochemistry, kidney

1. Introduction

Aquaporin 2 (AQP2) belongs to a large family of water channel proteins (WCPs), small tetrameric transmembrane proteins with a molecular mass of about 30 kDa. WCPs are better known as aquaporins (AQPs), as they have a specific three-dimensional structure with a pore that can be permeable to water and other small molecules, such as glycerol and urea (Nielsen et al., 2000; Fenton and Knepper, 2007; Benga, 2009; Gomes et al., 2009). The discovery of the first aquaporin in the human erythrocyte membrane by Peter Agre and coworkers in the 1980s revealed the previously unknown mechanism of rapid water flow across cell membranes (Agre et al., 1993). To date, at least 300 aquaporins have been discovered, and their presence has been confirmed in virtually all organisms examined. At least thirteen different aquaporin isoforms have been identified in mammals, and they are expressed in various fluid-transporting epithelial cells (Holmes, 2012). Aquaporins include three subfamilies: (1) aquaporins that are permeable only to water molecules (AQP0, AQP1, AQP2, AQP4, AQP5, and AQP6); (2) aquaglyceroporin, permeable to water and other small molecules (AQP3, AQP7, AQP8, AQP9, and AQP10); and (3) "unorthodox" aquaporins or "superaquaporins", which either have a low homology with conventional aquaporins or differ functionally from both aquaporins and aquaglyceroporins (AQP11 and AQP12) (Hub and Groot, 2008; Benga, 2009; Kortenoeven and Fenton, 2014).

The first aquaporin discovered, AQP2, was found in 1993 by Fushimi and coworkers in rat renal collecting tubules (Fushimi et al., 1993). In subsequent years, the presence of this protein was also confirmed in human and mouse kidneys. In the different sections of renal tubules 7 additional isoforms of AQPs (AQP1, AQP3, AQP4, AQP6, AQP7, AQP8, and AQP11) have been identified, but AQP2 is of particular importance for the regulation of water balance according to body needs. It is this protein that is fused to the apical plasma membrane in response to stimulation by antidiuretic hormone vasopressin (AVP) in the collecting duct principal cells, thereby increasing membrane permeability to water. As a result, low amounts of concentrated urine are excreted (Noda and Sasaki, 2005).

* Correspondence: katarzyna.michalek@zut.edu.pl

The great importance of AQP2 for renal water resorption and maintaining proper water balance in the body can be seen in patients suffering from nephrogenic diabetes insipidus (NDI). In the course of the first or second type of NDI resulting from mutations in the V2R gene (the second type of receptor for vasopressin) or mutation in a gene encoding AQP2, a loss of water retention in the renal collecting tubules occurs, which leads to increased excretion of large volumes of unconcentrated urine (Nielsen et al., 2002; Boone and Deen, 2008; Holmes, 2012).

The essential role played by AQP2 in fluid balance and urine concentration as well as its significant involvement in cell migration and epithelial morphogenesis has caused increased interest in this protein. The studies conducted in humans and laboratory animals have been extended to other species. The presence of this protein in the kidney and its potential role in renal water retention has been confirmed in animals such as monkeys, dogs, rabbits, cattle, horses, and mini pigs (Loffing et al., 2000; Floyd et al., 2007; Bauchet et al., 2011; Mobasheri et al., 2011; Michałek et al., 2014; Lou et al., 2014). To date, however, this type of study has not been performed in free-living animals, including wild boars.

Sus scrofa, the wild boar, is a species of large land mammal of the order *Artiodactyla*. In Europe it is the only suid species living in the wild. The current natural range of the wild boar is primarily the northern areas of Africa and central and southern Eurasia. Wild boars are omnivorous animals, but 90% of their food comes from plants, and approximately 10% is of animal origin (Pilarczyk et al., 2010). The suids (*Suidae*) have multipyramidal (multipapillary) kidneys but without the external lobation typically found in the bovine species. Some pyramids are simple, while others, as a result of fusion of two or more primitive separate pyramids, are compound. The apical portion of the pyramid, called the papilla, projects into the renal pelvis or its ramifications, the latter referred to as calyces. Papillae of simple pyramids are narrow and conical, whereas the compound pyramids, often located in the area of the renal poles, are broad. The suids have approximately 8–12 papillae per kidney. Porcine kidneys contain well over 1 million nephrons. Collecting ducts of the kidney have their openings at the tips of the papillae (Drolet, 2012). The daily urine output of *Sus scrofa* depends on many factors, among which diet, fluid intake, ambient temperature and humidity, and size and weight of the animal should be listed. The mean specific gravity of urine in adult swine is about 1.020, one of the lowest in comparison to farm animals (Ruckebusch et al., 1991), while the maximum urine concentration is about 1.1 osmol/L (Ketz, 1960). We think that, as in humans and other animal species, AQP2 plays a key role in water reabsorption in wild boar kidneys. In the literature there are no data concerning this protein in free-living *Sus scrofa* and so we conducted a study aimed at: (i) identification and (ii) localization of AQP2 in the kidneys of this animal species.

2. Materials and methods

We analyzed six kidneys obtained from 2- to 3-year-old male wild boars from northwestern Poland. Wild boars were shot during the 2013–2014 hunting season. Immediately after shooting, the kidneys were removed, cut into blocks, fixed in 4% buffered formalin, embedded in paraffin blocks, and sectioned at 2 μm on a rotary microtome. To conduct morphological and histochemical studies, sections were stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Sections were examined using an Olympus BX41 light microscope coupled to an Olympus digital camera (Olympus Optical Co., Tokyo, Japan).

For immunohistological studies, sections were deparaffinized using xylene, then rehydrated through graded ethanols. Sections were boiled in a Target Retrieval Solution (DAKO, Denmark) with pH 9.0. The activity of endogenous peroxidase was blocked by peroxidase blocking solution (Dako, Denmark) for 10 min in a humid chamber. Subsequently, the sections were incubated in a humid chamber with rabbit polyclonal primary antibody anti-AQP2 H7661 (Department of Biomedicine, Aarhus University, Denmark) diluted 1:900 in PBS with 0.1% BSA and 0.3% Triton X-100 overnight at 4 °C. Next, the sections were incubated with complex-containing secondary antibody conjugated with horseradish peroxidase (Dako REAL EnVision detection system peroxidase/DAB+, rabbit/mouse; Dako, Denmark). For the visualization immunohistochemical reaction DAB (Dako, Denmark) was used. The sections were counterstained with hematoxylin, dehydrated, and coverslipped. After each step in the above procedure, the sections were rinsed with PBS. They were examined using an Olympus BX41 light microscope coupled to an Olympus digital camera (Olympus Optical Co., Tokyo, Japan). Specificity of immunostaining was confirmed by following the above procedures in the absence of the primary antibody (negative control).

3. Results

Figures 1A–1J present the results obtained. In the preparations stained with H&E and PAS, normal and typical structures of the renal cortex and medulla were found in the animals examined (Figures 1A–1E). Glomeruli, proximal tubules with the brush border directed into the lumen, and distal tubules devoid of the brush border were observed in the renal cortex (Figures 1A, 1B, and 1D).

In addition, medullary rays were also visible penetrating through the renal cortex (Figure 1B). Collecting ducts with high epithelium and wide lumen were found in the medulla of the wild boar kidneys (Figures 1C and 1E).

Figures 1F–1I present immunoperoxidase labeling for aquaporin 2 in the sections of paraffin-embedded kidneys from wild boars. The presence of AQP2 was found both in the collecting ducts of medullary rays penetrating the renal cortex (Figures 1F and 1G) and in the collecting ducts of renal medulla (Figures 1H and 1I) of the kidneys. Strong staining of aquaporin 2 is mainly visible in the apical membrane of the collecting duct principal cells. Weak immunohistochemical staining was also present in the intracellular vesicles and the basolateral membrane (Figure 1I).

4. Discussion

The results of the current study clearly confirmed the presence of AQP2 in the principal cells of kidney collecting ducts in wild boards. Comparable results have been reported in the kidneys of rats, mice, rabbits, dogs, monkeys, horses, and mini pigs (Nielsen et al., 1993; Kishore et al., 1996; Loffing et al., 2000; Rojek et al., 2006; Eskild-Jedsen et al., 2007; Floyd et al., 2007; Bauchet et al., 2011). To our knowledge, the present study is the first report of AQP2 localization and expression in the kidneys of *Sus scrofa*.

AQP2 was immunolocalized in transverse sections of the renal cortex and medulla only in the cuboidal epithelium cells of the collecting ducts in the medullary rays and medullary layer of the wild boar kidneys. Particularly strong immunoreactions were observed in the apical membrane of the collecting duct cells. It is generally known that in mammals AVP is the main factor inducing the increase in AQP2 expression in the apical plasma membrane of the collecting duct cells. Vasopressin is released from the posterior pituitary under the increase of plasma osmolality (Sasaki, 2012; Takata et al., 2008). In the absence of vasopressin stimulation, aquaporin 2 is mainly localized in the endosomal vesicles of the trans-Golgi network (Moeller et al., 2012). Binding of AVP to vasopressin type 2 receptor (V2R), located on the basolateral membrane of the collecting duct principal cells, activates two type III and IV adenylate cyclases (AC)

Figure 1. Renal histology and immunohistochemical localization of AQP2 in the kidneys of wild boars (*Sus scrofa*). A, B, C – H&E staining. A– Renal cortex; glomeruli (black arrow), proximal tubules (P), distal tubules (D). B – Renal cortex; medullary rays (black arrow). C – Renal medulla; collecting ducts (CD). D, E – PAS staining. D – Renal cortex; glomeruli (black arrow), proximal tubules (P), distal tubules (D). E – Renal medulla; collecting ducts (CD). F, G, H, I – Immunolocalization and immunoexpression of AQP2. F, G – Renal cortex; expression of AQP2 in the medullary rays (black arrow). H – Renal medulla; expression of AQP2 in the collecting ducts (CD). I – Renal medulla; strong expression of AQP2 in the apical plasma membrane of the collecting duct principal cells (black arrow head). Weak expression of AQP2 in the intracellular vesicles (red arrow) and basolateral membrane (black arrow). J – Renal medulla; in the absence of the primary antibody no reaction product is observed (negative control).

(Hoffert et al., 2008; Wilson et al., 2013). This, in turn, results in an increase in intracellular cyclic adenosine monophosphate (cAMP) levels and leads to activation of protein kinase A (PKA), protein kinase B (AKT), serum/glucocorticoid-regulated kinase (Sgk), myosin light chain kinase, calmodulin-dependent kinase, mitogen-activated protein kinases, and intracellular Ca^{2+} oscillations (Fenton and Knepper, 2007; Wilson et al., 2013). This complex and not yet fully elucidated intracellular signaling mechanism, associated with the V2R stimulation, leads to the transport and subsequent fusion of AQP2 into the apical plasma membrane.

The active form of PKA phosphorylates Ser256, which is located in the cytoplasmic C-terminal region of AQP2 monomers. However, this site is also a substrate for other basophilic protein kinases (Wilson et al., 2013). Hoffert et al. (2008) stated that phosphorylation of at least three AQP2 monomers is necessary to start its translocation from vesicles and the consequent increase in the expression of this protein in the apical plasma membrane. The latest research in laboratory animals showed that the following serine sites were also phosphorylated: Ser261, Ser264, and Ser269 (Fenton et al., 2008; Hoffert et al., 2008). The level of serine phosphorylation in response to vasopressin increased at sites 256, 264, and 269, while it decreased at Ser261. The level of phosphorylated Ser261 is high without AVP stimulation (Moeller and Fenton, 2012). The function of phosphorylation at these sites has not been fully explained. However, it has been proved that phosphorylation of Ser256 is essential for the trafficking and fusion of AQP2 with the apical plasma membrane. It has also been found that different AQP2 phosphoforms are located in various intracellular organelles. AQP2 phosphorylated at Ser269 (AQP2pS269) was only detected in the apical membrane and was not observed in any intracellular organelles (Moeller et al., 2009). AQP2pS256 was found both in the apical membrane and intracellular vesicles (Christensen et al., 2000). Phosphorylated Ser261 (AQP2pS261) was mainly observed in the intracellular components of the Golgi apparatus and lysosomes (Hoffert et al., 2007). AQP2 phosphorylated at S264 is present in the apical and basal cellular membranes (Fenton et al., 2008).

In our study, anti-total AQP2 antibodies (antibodies binding all four phosphoforms of AQP2) were used for the immunocytochemical analysis of aquaporin 2 in the wild boar kidneys; thus, it was not possible to assess the location of particular AQP2 phosphoforms in the specific structures of collecting duct principal cells. However, it can be presumed on the basis of the cited literature that the strong expression observed in the apical membrane of collecting duct cells in the wild boar kidneys was associated with AQP2p256, AQP2pS264, and AQP2pS269.

Weak expression of AQP2 in the analyzed kidney preparations was also observed in a number of intracellular components. These immunoreactions were probably related to the presence of AQP2pS256 and AQP2pS61 in the intracellular vesicles. Intracellular trafficking between the apical plasma membrane and subapical vesicles determines the water permeability of principal cells in the collecting ducts (Yamamoto et al., 1995).

Nielsen et al. (1993), in their study on location and analysis of AQP2 expression in the rat kidney, claimed that a small amount of this protein can also be found in the basolateral membrane of the collecting duct cells, especially in the inner medullary collecting duct principal cells. Weak, positive staining for AQP2 along the basolateral membrane of collecting duct cells was also reported by Fushimi et al. (1993), Marples et al. (1995), and Michałek et al. (2014). According to these authors, the location of AQP2 in the basolateral membrane supports an exit pathway for the water across this membrane where abundant expression of AQP3 and AQP4 is also observed. Similar results were obtained in our research. We have found a weak but very distinct staining of AQP2 on the basolateral membrane of the principal collecting ducts of the wild boar kidneys. The results of the present study clearly indicate that in this species AQP2 plays an important role not only in the transport of water from the luminal fluid into the cell but also from the cell into the surrounding interstitial tissue. However, to fully explain the participation of AQP2 in the regulation of water balance in *Sus scrofa*, more detailed analyses are required. Unfortunately, conducting this type of research is difficult due to the wild nature of these animals. Nevertheless, we hope that the results of our work will be a contribution to the development of knowledge in this field.

In summary, AQP2 in wild boars (*Sus scrofa*) is located in the kidney principal collecting duct cells. The presence of AQP2 was confirmed mainly in the apical plasma membrane but also in the intracellular components and basolateral membrane of the principal cells of the collecting ducts. Due to the key role of AQP2 in the regulation of water balance in mammals and the lack of studies in the literature concerning the analysis of this protein in wild boars, this work completes the present state of knowledge by providing new information on immunolocalization and immunoexpression of aquaporin 2 in *Sus scrofa* kidneys.

References

Agre P, Preston GM, Smith BL, Jung JS, Raina S, Moon C, Guggino WB, Nielsen S (1993). Aquaporin chip: the archetypal molecular water channel. Am J Physiol Renal Physiol 265: F463–F476.

Bauchet AL, Masson R, Guffroy M, Slaoui M (2011). Immunohistochemical identification of kidney nephron segments in the dog, rat, mouse, and cynomolgus monkey. Toxicol Pathol 39: 1115–1128.

Benga G (2009). Water channel proteins (later called aquaporins) and relatives: past, present, and future. IUMB Life 6: 112–133.

Boone M, Deen P (2008). Physiology and pathophysiology of the vasopressin- regulated renal water reabsorption. Eur J Physiol 456: 1005–1024.

Christensen BM, Zelenina M, Aperia A, Nielsen S (2000). Localization and regulation of PKA-phosphorylated AQP2 in response to V(2)-receptor agonist/antagonist treatment. Am J Physiol Renal Physiol 278: F29–42.

Drolet R (2012). Urinary system. In: Zimmerman JJ, Karriker LA, Ramirez A, Schwartz KJ, Stevenson GW, editors. Disease of Swine. 10th ed. Oxford, UK: Wiley-Blackwell, pp. 263–283.

Eskild-Jedsen A, Paulsen LF, Wogensen FL, Olesen P, Pedersen L, Frøkiær J, Nyengaard JR (2007). AT_1 receptor blockade prevents interstitial and glomerular apoptosis but not fibrosis in pigs with neonatal induced partial unilateral obstruction. Am J Physiol Renal Physiol 292: F1771–F1781.

Fenton R, Moeller HB, Hoffert DJ, Yu MJ, Nielsen S, Knepper M (2008). Acute regulation of aquaporin-2 phosphorylation at Ser-264 by vasopressin. Proc Natl Acad Sci USA 105: 3134–3139.

Fenton RA, Knepper MA (2007). Mouse models and the urinary concentrating mechanism in the new millennium. Physiol Rev 87: 1083–1112.

Floyd RV, Mason SL, Proudman CJ, German AJ, Marples D, Mobasheri A (2007). Expression and nephron segment-specific distribution of major renal aquaporins (AQP1-4) in *Equus caballus*, the domestic horse. Am L Physiol Integr Comp Physiol 293: 492–503.

Fushimi K, Uchida S, Hara Y, Hirata Y, Marumo F, Sasaki S (1993). Cloning and expression of apical membrane water channel of rat kidney collecting tubule. Nature 361: 549–552.

Gomes G, Agasse A, Thiébaud P, Delrot S, Gerós H, Chaumont F (2009). Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. Biochim Biophys Acta 1788: 1213–1228.

Hoffert JD, Fenton RA, Moeller HB, Simons B, Tchapyjnikov D, McDill BW, Yu MJ, Pisitkun T, Chen F, Knepper MA (2008). Vasopressin-stimulated increase in phosphorylation at Ser^{269} potentiates plasma membrane retention of aquaporin-2. J Biol Chem 36: 24617–24627.

Hoffert JD, Nielsen J, Yu MJ, Pisitkun T, Schleicher SM, Nielsen S, Knepper MA (2007). Dynamics of aquaporin-2 serine-261 phosphorylation in response to short-term vasopressin treatment in collecting duct. Am J Physiol Renal Physiol 292: F691–700.

Holmes R (2012). The role of renal water channels in health and disease. Mol Aspects Med 33: 547–552.

Hub JS, Groot B (2008). Mechanism of selectivity in aquaporins and aquaglyceroporins. Proc Natl Acad Sci USA 105: 1198–1203.

Ketz HA (1960). Comparative studies on renal function in domestic animals. Arch Exp Vet Med 14: 411–419.

Kishore BK, Terrsi JM, Knepper MA (1996). Rat renal arcade segment expressed vasopressin-regulated water channel and vasopressin V_2 receptor. J Clin Invest 97: 2733–2771.

Kortenoeven LA, Fenton RA (2014). Renal aquaporins and water balance disorders. Biochim Biophys Acta 1840: 1533–1549.

Loffing J, Loffing-Cueni D, Macher A, Hebert SC, Olson B, Knepper MA, Rossier BC, Kaissling B (2000). Localisation of epithelial sodium channel and aquaporin-2 in rabbit kidney cortex. Am J Physiol Renal Physiol 278: F530–F539.

Lou W, Zhanjun L, Huang Y, Han Y, Yao Ch, Duan X, Ouyang H, Li L (2014). Generation of AQP2-Cre transgenic mini-pigs specifically expressing Cre recombinase in kidney collecting duct cells. Transgenic Res 23: 365–375.

Marples D, Knepper MA, Christensen EI, Nielsen S (1995). Redistribution of aquaporin-2 water channels induced by vasopressin in rat kidney inner medullary collecting duct. Am J Physiol Cell Physiol 269: 655–664.

Michałek K, Laszczyńska M, Ciechanowicz AK, Herosimczyk A, Rotter I, Oganowska M, Lepczyński A, Dratwa-Chałupnik A (2014). Immunohistochemical identification of aquaporin 2 in the kidneys of young beef cattle. Biotech & Histochem 89: 342–347.

Mobasheri A, Kendall BH, Maxwell JEJ, Sawran AV, German AJ, Marples D, Luck M, Royal MD (2011). Cellular localization of aquaporins along the secretory pathway of the lactating bovine mammary gland: an immunohistochemical study. Acta Histochem 113: 137–149.

Moeller HB, Fenton RA (2012). Cell biology of vasopressin-regulated aquaporin-2 trafficking. Eur J Physiol 464: 133–144.

Moeller HB, Knepper MA, Fenton RA (2009). Serine 269 phosphorylated aquaporin-2 is targeted to the apical membrane of collecting duct principal cells. Kidney Int 3: 295–303.

Nielsen S, DiGiovani SR, Christensen EI, Knepper MA, Harris HW (1993). Cellular and subcellular immunolocalization of vasopressin regulated water channel in rat kidney. Proc Natl Acad Sci USA 90: 11663–11667.

Nielsen S, Frøklær J, Marples D, Kwon T, Agre P, Knepper M (2002). Aquaporins in the kidney. From molecules to medicine. Physiol Rev 82: 205–244.

Nielsen S, Kwon T, Frøkiær J, Knepper MA (2000). Key roles of renal aquaporins in water-balance and water-balance disorders. News Physiol Sci 15: 136–143.

Noda Y, Sasaki S (2005). Trafficking mechanism of water channel aquaporin-2. Biol Cell 97: 885–892.

Pilarczyk B, Hendzel D, Pilarczyk R, Tomza-Marciniak A, Błaszczyk B, Dąbrowska-Wieczorek M, Bąkowska M, Adamowicz E, Bujak T (2010). Liver and kidney concentrations of selenium in wild boars (*Sus scrofa*) from northwestern Poland. Eur J Wild Res 56: 797–802.

Rojek A, Füchtbauer E, Kwon T, Frøkiær J, Nielsen S (2006). Severe urinary concentrating defect in renal collecting duct-selective AQP2 conditional-knockout mice. Proc Natl Acad Sci USA 15: 6037–6042.

Ruckebusch Y, Phaneuf LP, Dunlop R (1991). The urinary collecting and voiding system. In: Ruckebusch Y, Phaneuf LP, Dunlop R, editors. Physiology of Small and Large Animals. 1st ed. Philadelphia, PA, USA: BC Decker, pp. 184–188.

Sasaki S (2012). Aquaporin 2: from its discovery to molecular structure and medical implications. Mol Aspects Med 33: 535–546.

Takata K, Matsuzaki T, Tajika Y, Ablimit A, Hasegawa T (2008). Localization and trafficking of aquaporin 2 in the kidney. Histochem Cell Biol 130: 197–209.

Wilson JLL, Miranda CA, Knepper MA (2013). Vasopressin and the regulation of aquaporin-2. Clin Exp Nephrol 6: 751–764.

Yamamoto T, Sasaki S, Fushimi K, Kawasaki K, Yaoita E, Oota K, Hirata Y, Marmuro F, Kihara I (1995). Localization and expression of a collecting duct water channel, aquaporin, in hydrated and dehydrated rats. Exp Nephrol 3: 193–201.