Genetics and Molecular Research

Age-related changes in renal AQP3 and AQP4 expression in Sprague Dawley rats

X.H. Jing[1], J. Liu[2], W.Y. Hou[2] and Y. Gao[1]

[1]Department of Physiology, Zunyi Medical College Zhuhai Campus, Zhuhai, Guangdong, China
[2]Key Lab for Pharmacology of Ministry Education, Zunyi Medical College, Zunyi, Guizhou, China

Corresponding author: Y. Gao
E-mail: 705888097@qq.com

Genet. Mol. Res. 15 (3): gmr.15037532
Received August 27, 2015
Accepted February 11, 2016
Published August 5, 2016
DOI http://dx.doi.org/10.4238/gmr.15037532

Copyright © 2016 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution ShareAlike (CC BY-SA) 4.0 License

ABSTRACT. Aquaporin (AQP) 3 and AQP4 are important in urine concentrating mechanisms and in other physiological functions such as brain water balance, cell migration, cell proliferation, fat metabolism, and epidermal hydration. The results of studies investigating AQP3 and AQP4 expression in the kidneys are inconsistent, and systematic research is rare. This study aimed to obtain a better understanding of the changes in renal AQP3 and AQP4 mRNA expression that take place with age. The expression of AQP3 and AQP4 mRNA, during prenatal and postnatal development, and during aging, was investigated in kidneys from Sprague-Dawley rats. The pattern of AQP3 expression was similar to that of AQP4 expression during development, and both were detected at gestational day 19 in the rat kidney where they maintained a stable level to postnatal day 14. Subsequently, a significant increase in expression was observed from day 21 to day 35, with peak expression occurring at day 35. No significant change in AQP3 or

AQP4 mRNA expression was observed after day 35, apart from AQP4, which increased at day 540. Moreover, the expression of both AQP3 and AQP4 on day 850 was higher than on day -2, and lower than on days 28 and 35. The expression of AQP3 and AQP4 was similar on days 1, 7, 14, and 21. These findings indicate that mRNA expression of AQP3 and AQP4 varies with age, which should be considered when treating kidney disease in pediatric and elderly patients.

Key words: Age-related; Kidney; AQP3; AQP4; Sprague Dawley rat

INTRODUCTION

The kidneys are particularly affected by ageing, even in the absence of other pathologies (Martin and Sheaff, 2007; Percy et al., 2008). Decreased renal function and increased susceptibility to chronic kidney diseases are observed in aging kidneys, primarily due to glomerulosclerosis, tubular atrophy, interstitial fibrosis, and loss of functional renal mass (Martin and Sheaff, 2007; Silva et al., 2010) Aquaporins (AQPs) are a family of small membrane proteins of approximately 30 kDa that serve as semi-permeable channels. AQPs are also called major intrinsic proteins, which facilitate water transport across biological membranes (Xing et al., 2014), and are involved in diverse physiological functions including brain water balance, cell migration, cell proliferation, neuro-excitation, fat metabolism, and epidermal hydration (Tradtrantip et al., 2009). To date, 13 members of the AQP family belonging to three groups have been identified: water-selective channels; aquaglyceroporins, which transport water, glycerol, as well as urea; and unorthodox channels (Poling et al., 2014).

Three AQPs (AQP2, AQP3, and AQP4) are expressed in the kidney collecting duct (Yamamoto et al., 1997; Nejsum et al., 2001; Bonilla-Felix, 2004; Kortenoeven and Fenton, 2014) AQP2 is abundant in sub-apical vesicles, in the apical plasma membrane of the principal connecting duct, and in inner medullary duct cells. AQP2 is the main target of vasopressin (AVP), which regulates water permeability in the collecting duct (Nejsum et al., 2001; Bonilla-Felix, 2004). AVP regulates AQP2 via short-term and long-term regulatory mechanisms, which are fundamentally different (Nielsen and Agre, 1995; Poulsen et al., 2013) AQP2 null mice fail to thrive and die postnatally as a result of excessive extracellular fluid loss, indicating that the concentration of urine is dependent on the presence of AQP2 (Tamma et al., 2012; Xing et al., 2014). AQP3 and AQP4 are localized to the basolateral plasma membranes of principal cells in the connecting tubule and collecting duct (Murillo-Carretero et al., 1999; Nielsen et al., 1999; 2002; Tamma et al., 2012; Kortenoeven and Fenton, 2014) and provide potential pathways of water reabsorption via AQP2 (Wang et al., 2002; Nishimura and Yang, 2013; Koetenoeven and Fenton, 2014; Marlar et al., 2014). AQP3 is located in the cortical and outer medullary collecting ducts and AQP4 is located in the inner medullary collecting duct membrane (Terris et al., 1995; Verkman, 1998; Nielsen et al., 2002; Kim et al., 2005; Verkman, 2006; Nishimura and Yang, 2013) AQP3 is regulated by long-term AVP stimulation (Terris et al., 1995; Yang et al., 2013; Xing et al., 2014) and extracellular pH (Zelenina et al., 2003; Castle, 2005). In addition to water, AQP3 also transports glycerol and urea (King and Agre, 1996; Lee et al., 1997; Kuwahara et al., 1997) AQP4 enables water permeation in the basolateral plasma membrane of the rat inner medullary collecting duct (Ma et al., 2000). Deletion of AQP3 in mice leads to polyuria and down regulation of AQP2 (Ma et al., 2000;

Tamma et al., 2012) because of severe defects in the urinary concentrating ability. Conversely, AQP4 null mice show only a mild deficit in urinary concentrating ability. In addition, AQP3/AQP4 double-knockout mice show more severe polyuria than single AQP3 knockout mice (Ma et al., 2000; Tamma et al., 2012; Marlar et al., 2014), indicating the existence of a compensatory mechanism between AQP3 and AQP4 (Marlar et al., 2014).

Previous studies investigating the expression of renal AQP3 and AQP4 mRNA from gestational day 15 to 12 months of age, particularly in gestational and perinatal rats, have provided inconsistent results (Yamamoto T et al., 1997). Little is known about the expression patterns of AQP3 and AQP4 mRNA after 12 months. To better understand the expression profiles of AQP3 and AQP4, the present study was designed to investigate the renal expression of AQP3 and AQP4 mRNA during development in Sprague Dawley (SD) rats.

MATERIAL AND METHODS

Animals and sample collection

SD rats (250-300 g) were obtained from the Experimental Animal Center of Third Military Medical College (Chongqing, China; CXK2007-005). Rats were housed in SPF-grade animal facilities (Certificate No. SYXK 2011-004) at Zunyi Medical College, under controlled conditions (12-h light/dark cycle at 22° ± 1°C, 50% humidity) with free access to purified water and standard rat chow. All animal care and experimental protocols followed the Animal Management Guidelines of the Chinese Ministry of Health and were approved by the Institutional Animal Use and Care Committee of Zunyi Medical College.

Adult male and female SD rats aged 10 weeks were mated overnight. The presence of a vaginal plug was designated as day (GD 0) of gestation. The kidneys were collected on GD 19 (day -2), on developmental days (days 1, 7, 14, 21, 28, 35, and 60) and on days of advancing age (days 180, 420, 540, and 850). From day -2 to 180, rats were classified as offspring; from day 420 to 850, rats were classified as additional age-matched rats. Kidneys were stored at -80°C prior to analysis and total RNA was subsequently extracted from each kidney.

RNA isolation and real-time PT-PCR analysis

Approximately 50-100 mg of kidney tissue was homogenized in 1 mL TRIzol (TaKaRa Biotechnology, Dalian, China). The quality and quantity of RNA were determined by the 260/280 ratio, which was between 1.8 and 2.0 for all samples. Total RNA was reverse transcribed using the High Capacity Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA). Primer3 software was used to design primers, and sequences are listed in Table 1. IQ™ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) was used for real-time RT-PCR. The relative expression of genes of interest was calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to the house-keeping gene *GAPDH*.

Table 1. Primer sequences used for real-time RT-PCR analysis.

Gene	Forward	Reverse
GAPDH	5′-ggCACAgTCAAggCTgAgAATg-3′	5′-ATggTggTgAAgACgCCAgTA-3′
AQP3	5′-AAgCTgCCCATCTACACACT-3′	5′-gATACCAgCTgTgCCATTgg-3′
AQP4	5′-TCCCTCTgCTTTggACTCAg-3′	5′-gCgATgCTgATCTTTCgTgT-3’

Statistical analysis

Arithmetic means and standard errors of means are presented. Statistical analyses were implemented using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test for multiple comparisons with SPSS13.0 software. P values lower than 0.05 were considered to be significantly different.

RESULTS

AQP3 mRNA expression in the kidney

The pattern of AQP3 mRNA expression changed with age, with expression being the lowest on day -2. There was no difference in the level of mRNA expression on days 1, 7, and 14 compared with that on day -2, while on days 21, 28, and 35, AQP3 expression was significantly higher than on day 14, with the largest change observed at day 35. At day 60, expression was significantly lower than at day 35, but was not different to that observed on days 21, 180, 420, 540, and 850. There was no difference in AQP3 expression on day 180 and 420 compared with that on day 60, and subsequently, expression at day 540 was significantly higher than that at day 420, before decreasing until day 850. Expression of AQP3 was significantly higher at day 850 than at day -2, with meaningful lower than on days 28, 35, and 540, and was indistinguishable from that on days 1, 7, 14, and 21 (Figure 1).

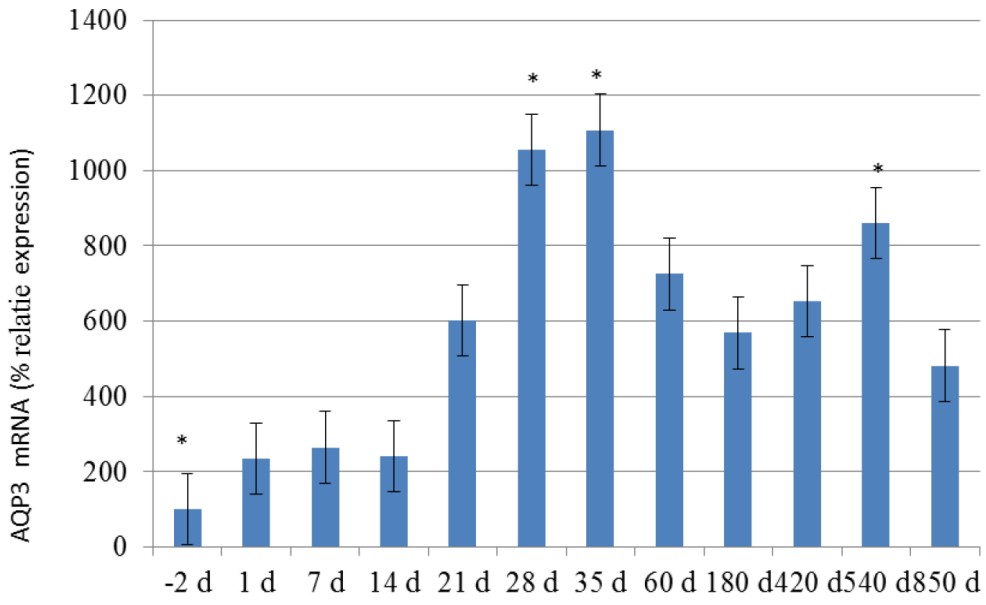

Figure 1. Age-related variation in AQP3 mRNA expression in kidneys from Sprague-Dawley AQP3 expression on days -2 (N = 6), 1 (N = 6), 7 (N = 6), 14 (N = 6), 21 (N = 6), 28 (N = 6), 35 (N = 6), 60 (N = 6), 180 (N = 9), 420 (N = 4), 540 (N = 11), and 850 (N = 9) were assayed by RT-PCR. The relative expression at each point was normalized to that of GAPDH. *Statistically significant differences relative to day 850 are indicated as $P < 0.05$.

AQP4 mRNA expression in the kidney

The pattern of AQP4 mRNA expression changed with age, and was lowest at day -2, with no difference observed on days 1, 7, and 14. AQP4 mRNA levels were higher on days 21, 28, and 35 than on day 14, with peak expression observed on day 35. Expression then declined until day 60. AQP4 mRNA levels on days 180, 420, 540 and 850 were not different from that on day 60, and there was no difference between expression on day 60 and that on days 21, 180, 420, 540, and 850. Expression on day 850 was significantly higher than on day -2, lower than on days 28 and 35, and was similar to that observed on days 1, 7, 14, and 21 (Figure 2). Expression at day 850 of AQP4 showed little variation from that on days 1, 7, 14, and 21, but never reached statistical difference.

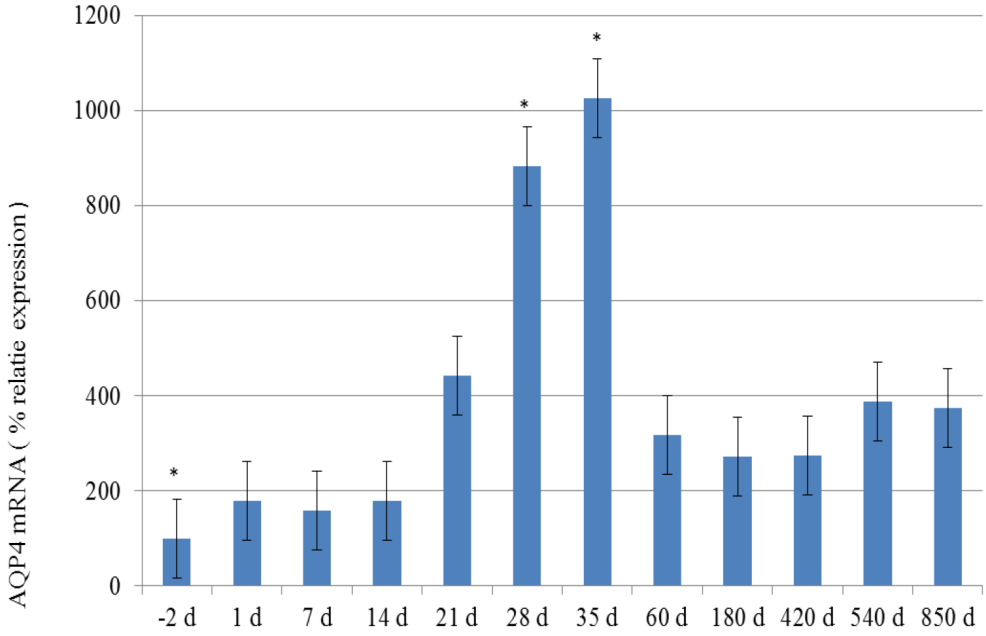

Figure 2. Age-related variation *in AQP4* mRNA expression in kidneys from Sprague-Dawley rats. AQP4 expression on days -2 (N = 6), 1 (N = 6), 7 (N = 6), 14 (N = 6), 21 (N = 6), 28 (N = 6), 35 (N = 6), 60 (N = 6), 180 (N = 9), 420 (N = 4), 540 (N = 11), and 850 (N = 9) were assayed by RT-PCR. The relative expression at each point was normalized to that of GAPDH. *Statistically significant differences relative to day 850 are indicated as $P < 0.05$.

DISCUSSION

AQP3 and AQP4 are constitutively expressed water channels involved in the concentration of urine. Therefore, insight into phylogenetic and physiological variations in the expression of AQP3 and AQP4 mRNA is important for understanding their function.

The results of the present study provide new information about AQP3 and AQP4 mRNA expression over time in the kidneys of SD rats. The pattern of AQP3 mRNA expression was similar to that of AQP4 throughout development until day 60.

AQP3 and AQP4 mRNA was detected in 19-day-old (day -2) fetal rat kidneys and was maintained at a constant level throughout the first three weeks postnatal. An increasing trend was observed from day 14, with the greatest change occurring on day 35. This is consistent with a previous report (Poling et al., 2014) in which significantly increased urine concentrating capacity was observed during the first 3.5 weeks of postnatal life, and subsequently decreased on day 60. In the present study, there was no difference in the expression of AQP3 and AQP4 on day 60 and on days 21, 180, 420, 540, and 850. In accordance with the reduction was along the opposite direction of growth where the level at day 60 dropped to the level at day 21. There was no difference in the expression of AQP3 on days 60, 180, and 420, whereas expression on day 540 was significantly higher than on day 420, and then dropped until day 850. This observation is interesting, and might be due to the sample itself or to the experimental conditions. There was no difference in the expression of AQP4 on days 60, 180, 420, 540, and 850. Moreover, both AQP3 and AQP4 were expressed at significantly higher levels on day 850 than on day-2D, and at lower levels than on days 28 and 35, with little variation from the expression levels observed on days 1, 7, 14, and 21. These data suggest that the level of AQP3 and AQP4 mRNA expression on day 850 was the same as that observed during the first three postnatal weeks.

These changes in expression correspond to age-related changes in the urinary concentrating capacity. AQP3 mRNA expression almost paralleled that of AQP4. A similar pattern of AQP3 expression has been found by other investigators, who reported that AQP3 could be detected in ureteric buds of the rat kidney as early as embryonic day 18 (Baum et al., 1998). Interestingly, Lee et al. (1997) reported that AQP3 mRNA could be detected at birth and that levels were stable after birth throughout life (Yamamoto et al., 1997; Xing et al., 2014) AQP4 mRNA was barely detectable in fetal rat kidneys, whereas it was detected after birth and was maintained at stable levels throughout life (Xing et al., 2014). Another study reported low levels of AQP4 mRNA expression in the kidneys from birth to 6-months of age, which were minimal at 12 months (Yamamoto et al., 1997). Discrepancies exist among results of studies investigating AQP3 and AQP4 mRNA expression during development. The discrepancy between our results and those from previous studies may be explained by differences in the experimental conditions, including the species studied (i.e., rat *vs* mouse).

In conclusion, expression of AQP3 and AQP4 mRNA in the kidney changed throughout the life of SD rats. These findings contribute to current knowledge on the renal expression of AQP3 and AQP4 in SD rats during gestational day 19 to postpartum day 850. These findings warrant further investigation into AQP3 and AQP4 biology to better understand variations in their gene expression, and to provide useful information that may assist in the development of therapies that effectively restore physiological disruptions associated with kidney disease.

Conflicts of interest

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Research funded by the National Natural Science Foundation of China (#31160214).

REFERENCES

Baum MA, Ruddy MK, Hosselet CA and Harris HW (1998). The perinatal expression of aquaporin-2 and aquaporin-3 in developing kidney. *Pediatr. Res.* 43: 783-790. http://dx.doi.org/10.1203/00006450-199806000-00011

Bonilla-Felix M (2004). Development of water transport in the collecting duct. *Am. J. Physiol. Renal Physiol.* 287: F1093-F1101. http://dx.doi.org/10.1152/ajprenal.00119.2004

Castle NA (2005). Aquaporins as targets for drug discovery. *Drug Discov. Today* 10: 485-493. http://dx.doi.org/10.1016/S1359-6446(05)03390-8

Kim SW, Gresz V, Rojek A, Wang W, et al. (2005). Decreased expression of AQP2 and AQP4 water channels and Na,K-ATPase in kidney collecting duct in AQP3 null mice. *Biol. Cell* 97: 765-778. http://dx.doi.org/10.1042/BC20040148

King LS and Agre P (1996). Pathophysiology of the aquaporin water channels. *Annu. Rev. Physiol.* 58: 619-648. http://dx.doi.org/10.1146/annurev.ph.58.030196.003155

Kortenoeven ML and Fenton RA (2014). Renal aquaporins and water balance disorders. *Biochim. Biophys. Acta* 1840: 1533-1549. http://dx.doi.org/10.1016/j.bbagen.2013.12.002

Kuwahara M, Gu Y, Ishibashi K, Marumo F, et al. (1997). Mercury-sensitive residues and pore site in AQP3 water channel. *Biochemistry* 36: 13973-13978. http://dx.doi.org/10.1021/bi9711442

Lee MD, King LS and Agre P (1997). The aquaporin family of water channel proteins in clinical medicine. *Medicine (Baltimore)* 76: 141-156. http://dx.doi.org/10.1097/00005792-199705000-00001

Ma T, Song Y, Yang B, Gillespie A, et al. (2000). Nephrogenic diabetes insipidus in mice lacking aquaporin-3 water channels. *Proc. Natl. Acad. Sci. USA* 97: 4386-4391. http://dx.doi.org/10.1073/pnas.080499597

Marlar S, Arnspang EC, Pedersen GA, Koffman JS, et al. (2014). Measuring localization and diffusion coefficients of basolateral proteins in lateral versus basal membranes using functionalized substrates and kICS analysis. *Biochim. Biophys. Acta* 1838: 2404-2411. http://dx.doi.org/10.1016/j.bbamem.2014.06.005

Martin JE and Sheaff MT (2007). Renal ageing. *J. Pathol.* 211: 198-205. http://dx.doi.org/10.1002/path.2111

Murillo-Carretero MI, Ilundáin AA and Echevarria M (1999). Regulation of aquaporin mRNA expression in rat kidney by water intake. *J. Am. Soc. Nephrol.* 10: 696-703.

Nejsum LN, Kwon TH, Marples D, Flyvbjerg A, et al. (2001). Compensatory increase in AQP2, p-AQP2, and AQP3 expression in rats with diabetes mellitus. *Am. J. Physiol. Renal Physiol.* 280: F715-F726.

Nielsen S and Agre P (1995). The aquaporin family of water channels in kidney. *Kidney Int.* 48: 1057-1068. http://dx.doi.org/10.1038/ki.1995.389

Nielsen S, Kwon TH, Christensen BM, Promeneur D, et al. (1999). Physiology and pathophysiology of renal aquaporins. *J. Am. Soc. Nephrol.* 10: 647-663.

Nielsen S, Frøkiaer J, Marples D, Kwon TH, et al. (2002). Aquaporins in the kidney: from molecules to medicine. *Physiol. Rev.* 82: 205-244. http://dx.doi.org/10.1152/physrev.00024.2001

Nishimura H and Yang Y (2013). Aquaporins in avian kidneys: function and perspectives. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 305: R1201-R1214. http://dx.doi.org/10.1152/ajpregu.00177.2013

Percy CJ, Power D and Gobe GC (2008). Renal ageing: changes in the cellular mechanism of energy metabolism and oxidant handling. *Nephrology (Carlton)* 13: 147-152. http://dx.doi.org/10.1111/j.1440-1797.2008.00924.x

Poling HM, Mohanty SK, Tiao GM and Huppert SS (2014). A comprehensive analysis of aquaporin and secretory related gene expression in neonate and adult cholangiocytes. *Gene Expr. Patterns* 15: 96-103. http://dx.doi.org/10.1016/j.gep.2014.05.003

Poulsen SB, Kim YH, Frøkiær J, Nielsen S, et al. (2013). Long-term vasopressin-V2-receptor stimulation induces regulation of aquaporin 4 protein in renal inner medulla and cortex of Brattleboro rats. *Nephrol. Dial. Transplant.* 28: 2058-2065. http://dx.doi.org/10.1093/ndt/gft088

Silva E, Pinto V, Simão S, Serrão MP, et al. (2010). Renal aging in WKY rats: changes in Na+,K+ -ATPase function and oxidative stress. *Exp. Gerontol.* 45: 977-983. http://dx.doi.org/10.1016/j.exger.2010.09.012

Tamma G, Procino G, Svelto M and Valenti G (2012). Cell culture models and animal models for studying the patho-physiological role of renal aquaporins. *Cell. Mol. Life Sci.* 69: 1931-1946. http://dx.doi.org/10.1007/s00018-011-0903-3

Terris J, Ecelbarger CA, Marples D, Knepper MA, et al. (1995). Distribution of aquaporin-4 water channel expression within rat kidney. *Am. J. Physiol.* 269: F775-F785.

Tradtrantip L, Tajima M, Li L and Verkman AS (2009). Aquaporin water channels in transepithelial fluid transport. *J. Med. Invest.* 56 (Suppl): 179-184. http://dx.doi.org/10.2152/jmi.56.179

Verkman ASM (1998). Role of aquaporin water channels in kidney and lung. *Am. J. Med. Sci.* 316: 310-320.

Verkman AS (2006). Roles of aquaporins in kidney revealed by transgenic mice. *Semin. Nephrol.* 26: 200-208.

Wang W, Li C, Kwon TH, Knepper MA, et al. (2002). AQP3, p-AQP2, and AQP2 expression is reduced in polyuric rats with hypercalcemia: prevention by cAMP-PDE inhibitors. *Am. J. Physiol. Renal Physiol.* 283: F1313-1325.

Xing L, Wen JG, Frøkiær J, Djurhuus JC, et al. (2014). Ontogeny of the mammalian kidney: expression of aquaporins 1, 2, 3, and 4. *World J. Pediatr.* 10: 306-312. http://dx.doi.org/10.1007/s12519-014-0508-7

Yamamoto T, Sasaki S, Fushimi K, Ishibashi K, et al. (1997). Expression of AQP family in rat kidneys during development and maturation. *Am. J. Physiol.* 272: F198-F204.

Yang M, Gao F, Liu H, Yu WH, et al. (2013). Hyperosmotic induction of aquaporin expression in rat astrocytes through a different MAPK pathway. *J. Cell. Biochem.* 114: 111-119. http://dx.doi.org/10.1002/jcb.24308

Zelenina M, Bondar AA, Zelenin S and Aperia A (2003). Nickel and extracellular acidification inhibit the water permeability of human aquaporin-3 in lung epithelial cells. *J. Biol. Chem.* 278: 30037-30043. http://dx.doi.org/10.1074/jbc.M302206200