

Down-regulation of Aquaporin 4 in Human Placenta Throughout Pregnancy

MARIA DE FALCO^{1*}, LUIGI COBELLIS^{2*}, MARCO TORELLA², GENNARO ACONE², LAURA VARANO³, ANNA SELLITTI¹, ANGELO RAGUCCI², GABRIELE COPPOLA⁴, ROBERTO CASSANDRO⁵, VINCENZA LAFORGIA¹, LORENZO VARANO¹ and ANTONIO DE LUCA⁴

¹*Department of Biological Sciences, Section of Evolutionary and Comparative Biology, University of Naples "Federico II", Naples;*

Departments of ²Gynecology, Obstetric and Reproductive Science and

⁴Medicine and Public Health, Section of Clinical Anatomy, Second University of Naples, Naples;

³IRCCS "Casa Sollievo della Sofferenza" Hospital, San Giovanni Rotondo (Foggia);

⁵San Giuseppe Hospital, Service of Pneumology, Milan, Italy

Abstract. *Background:* The family of mammalian aquaporins (AQP) consists of 12 known members, each with a specific tissue distribution and membrane localization pattern. AQP4 is the first member of this family identified in biological membranes. This water channel protein is primarily expressed in astrocytes but is also localized in ependymocytes and endothelial cells, suggesting its involvement in the movement of water between the blood and brain, and between the brain and cerebrospinal fluid (CSF). To date, the regulation of AQP4 expression in the human placenta has not been studied. The purpose of this work was to investigate AQP4 localization and expression in the human placenta during gestation. *Materials and Methods:* A total of 30 samples, 15 full-term placentae and 15 chorionic villous samples from first trimester, for the immunohistochemical analysis of AQP4 expression were used. The gestation period ranged from 5 to 40 weeks. *Results:* A decrease of AQP4 expression in the syncytiotrophoblast from the first to the third trimester of gestation, in contrast with an increased expression shown by endothelial cells and stroma of placental villi was found. *Conclusion:* Our results may suggest that AQP4-mediated maternal-fetal fluid exchange could play an important role in the control of ion homeostasis and water balance in the human placenta throughout pregnancy.

*Both authors contributed equally to this study.

Correspondence to: Dr. Antonio De Luca, Department of Medicine and Public Health, Section of Clinical Anatomy, Second University of Naples, Via L. Armanni 5, 80138 Naples, Italy. Fax: +39 081 458225, e-mail: antonio.deluca@unina2.it

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Over the past decade, a family of related water-transporting proteins called aquaporins (AQP) has been identified (1-3). They are a family of channel-forming transmembrane proteins that facilitate the movement of water, glycerol and other solutes across the plasma membrane of cells (4-8). The family of mammalian AQPs consists of 12 known members, each with a specific tissue distribution and membrane localization pattern (9), but many more from amphibians, plants, yeast, bacteria and other lower organisms have been cloned (3). AQPs are small (~30 kDa) hydrophobic proteins that assemble in membranes as tetramers. The aquaporins can be classified functionally into two groups: AQP1, AQP2, AQP4, AQP5 and AQP8 are mainly permeable to water; AQP3, AQP6, AQP7 and AQP9 are also water permeable, but in addition they have significant permeability to small solutes such as glycerol (AQP3, AQP7 [aquaglyceroporins]) and larger neutral solutes (AQP9), and, possibly under some conditions, to ions (AQP6). The sites of aquaporin expression suggest a role in renal water reabsorption, cerebrospinal fluid (CSF) dynamics, aqueous fluid dynamics, glandular secretion and other physiological processes (3).

AQP4 was the first aquaporin to be observed and identified in biological membranes (9). AQP4 was initially cloned from rat lung (10); subsequently isoforms from rat brain (11) and ortholog from human (12) and mouse (13) were sequenced (3). AQP4 is the most abundant water channel in brain, where it is mainly localized in pericapillary astrocyte foot processes, glial limiting membranes (comprising the outer brain surface and subependyma) and ependyma (14-16). Recently, AQP4 was also found in the brain vascular endothelium (8, 10, 11, 14, 17). This distribution suggests the involvement of AQP4 in the movement of water between the blood and brain and between the brain and CSF. Recent phenotype

studies in transgenic mice lacking AQPs 1-5 have been very informative (18, 19). For example, mice lacking AQP4 have impaired hearing (3, 20). The phenotype studies suggest that aquaporins are important for rapid, near-isosmolar fluid transport, as occurs in kidney proximal tubules and salivary glands, and for water transport when it is driven by established osmotic gradients, as in kidney collecting ducts. The phenotype studies have also shown that the tissue-specific expression of an aquaporin does not indicate physiological significance – for example, deletion of AQP4 in skeletal muscle and gastric parietal cells does not impair skeletal muscle function (21), or gastric acid production (3, 22).

Pregnancy has been shown to up-regulate aquaporin expression in other organs such as the uterus (8, 23, 24), but the regulation of AQP4 expression in the human placenta during pregnancy has not been studied. The placenta is composed of maternal and fetal material and plays an integral role not only in the supply of nutrients to the fetus but also in the maintenance of pregnancy (25). The human placenta is able to synthesize and secrete a variety of hormones and molecules that regulate the fine balance between proliferation, differentiation and invasion of trophoblastic cells. In order to better clarify AQP4 localization in human placenta, an immunohistochemical analysis of AQP4 expression throughout pregnancy in physiological conditions was carried out.

Materials and Methods

Samples. Human placental samples were obtained with informed consent from patients undergoing surgery such as cesarean section for normal placenta and uterine evacuation for normal chorionic villi. A total of 30 samples, 15 full-term placentae and 15 chorionic villous samples from the first trimester were used and the gestation period ranged from 5 to 40 weeks. The specimens were immediately fixed in formalin for immunohistochemistry.

Immunohistochemistry. Immunohistochemistry was carried out essentially as described previously (26). Briefly, sections embedded in paraffin from each specimen were cut at 5 µm, mounted on glass and dried overnight at 37°C. All sections were then deparaffinized in xylene, rehydrated through a graded series of alcohol and washed in phosphate-buffered saline (PBS). PBS was used for all subsequent washes and for antiserum dilution. Tissue sections were quenched sequentially in 3% hydrogen peroxide and blocked with PBS-6% non-fat dry milk (Bio-Rad, Hercules, CA, USA) for 1 h at room temperature. Slides were then incubated at 4°C overnight with rabbit polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) raised against AQP-4 (sc-6014) at a 1:400 dilution. After several washes (3x5 min) to remove excess antibody, the slides were incubated with diluted anti-goat biotinylated antibody, anti-rabbit biotinylated antibody or anti-mouse biotinylated antibody (Vector Laboratories, Burlingame, CA, USA) for 1 h. All the slides were then processed by the ABC method (Vector Laboratories) for 30 min at room temperature. Novared (Vector

Laboratories) was used as the chromogen and hematoxylin was used as nuclear counterstain. Negative controls for each tissue section were prepared by substituting the primary antiserum with non-immune IgG. For each experiment, all slides were stained in a single batch and thus received equal staining. Immunohistochemical staining intensity was evaluated by ranking: 0 (absent), 1 (weak), 2 (moderate) or 3 (intense) as described by Selam *et al.* (27). For each specimen, an HSCORE value was derived by summing the percentages of cells/areas that stained at each intensity and multiplying that by the weighted intensity of the staining. For example: HSCORE = $\Sigma P_i (i + 1)$ where i represents the intensity scores and P_i the corresponding percentage of cells/areas.

An average of 22 fields were observed for each tissue by three observers at different times and the average score was used. All values were expressed as mean ± standard error of mean (SEM) and differences were compared using Student's *t*-test.

Results

Distribution of AQP4 in human placenta during gestation. The localization and distribution of AQP4 in human placenta during gestation by immunohistochemistry was investigated. We observed that in the first trimester, AQP4 was localized in the cytoplasm of syncytiotrophoblast at an intense level of expression (Figure 1a). In contrast, the AQP4 expression in the cytotrophoblast was weak. In addition, we observed a moderate immunopositivity for AQP4 in the stroma of first trimester placental villi and in the cytoplasm of endothelial cells (Figure 1b). During the third trimester of gestation, AQP4 immunopositivity slightly decreased in the syncytiotrophoblast (Figure 1c), while AQP4 immunopositivity increased in the stroma of placental villi and in the cytoplasm of endothelial cells (Figure 1c). The AQP4 immunopositivity in the cytotrophoblast was always weak during the third trimester of gestation (Figure 1d).

In Figure 2 the expression pattern of AQP4 during pregnancy, as detected by immunohistochemical staining intensity analysis, is depicted. We observed a decrease of AQP4 expression from the first to the third trimester of gestation in the syncytiotrophoblast, in contrast with an increased expression of AQP4 in endothelial cells and stroma of placental villi. The AQP4 expression in the cytotrophoblast appeared unchanged from the first to the third trimester of gestation.

Discussion

AQP4 is expressed in astrocyte foot processes near blood vessels in rat (14, 15) and human (28) brain, as well as in ependymal and pial surfaces in contact with CSF (15, 29, 30). This localization suggests that AQP4 plays a critical role in brain water balance (3). AQP4 appears to be selective for the passage of water and is unique among the aquaporins in its exceptionally high intrinsic water

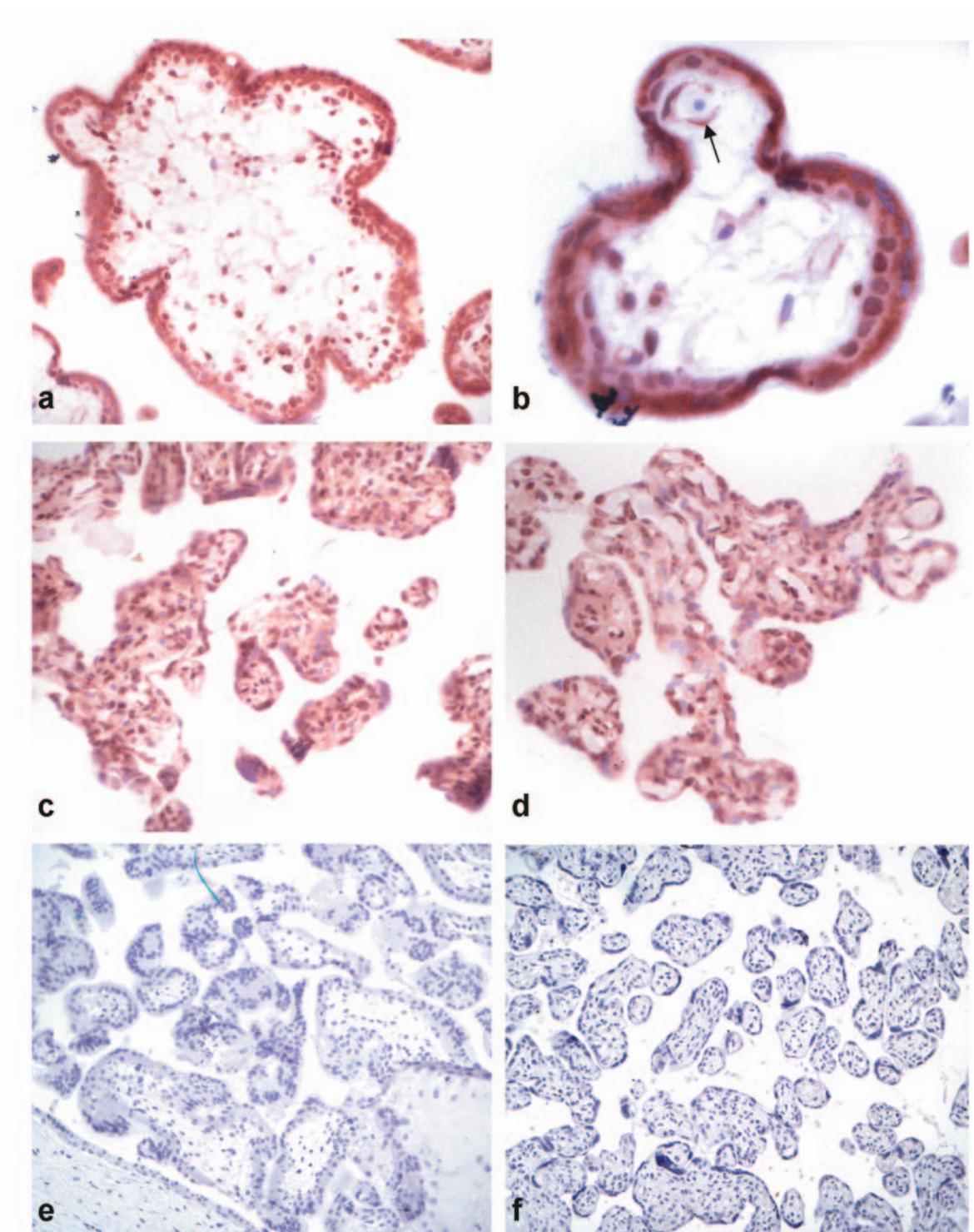


Figure 1. Localization of aquaporin 4 (AQP4) in human placenta throughout gestation. a) AQP4 localization in the first trimester of gestation showing the high immunopositivity in the syncytiotrophoblast, $\times 150$; b) First trimester placental villous showing the intense immunopositivity of AQP4 in the syncytiotrophoblast and the moderate positivity in the cytoplasm of endothelial cells (arrows), $\times 640$; c) AQP4 localization in the third trimester of gestation, $\times 150$; d) AQP4 moderate immunolocalization in the syncytiotrophoblast, stroma and endothelial cells of the third trimester placental villi, $\times 300$; e) Representative negative control of a placenta of the first trimester of gestation, $\times 100$; f) Representative negative control of a placenta of the third trimester of gestation, $\times 100$.

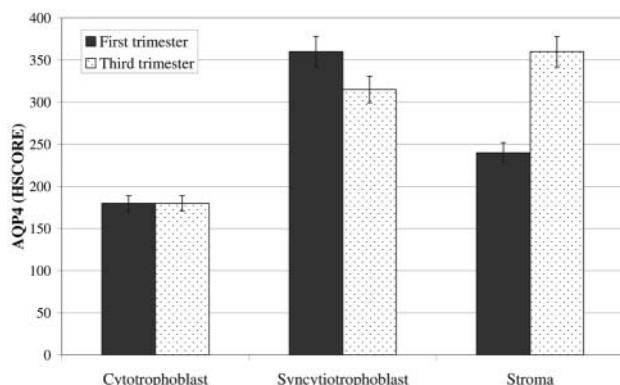


Figure 2. Gestational time course of cytотrophoblast, syncytiotrophoblast and stromal cell intensity staining for AQP4 in placental villi during the first and the third trimester of gestation, is shown. Vertical lines show S.E.M.

permeability (31) and its assembly in membranes in regular square arrays called orthogonal arrays of particles (OAPs) (3). AQP4 in astrocytes is thought to contribute to blood-brain barrier (BBB) properties by taking up excess water brought into the brain by disruption of the BBB (32). However, excessive AQP4 may be detrimental and promote edema formation. The involvement of AQP4 in brain edema formation has been demonstrated in studies using knockout mice (8). Another pathological condition in which AQP4 may be involved is in vasogenic edema formation during eclampsia. It is likely that the higher levels of AQP4 protein in the brain during pregnancy and postpartum do not cause edema under normal conditions, but they rather predispose the brain to greater edema formation when a stressor that disrupts the BBB, such as acute hypertension, is introduced (8). Along these lines, Belfort *et al.* showed that pre-eclamptic women with normal blood pressure had abnormally high cerebral perfusion pressure, suggesting that elevated intravascular pressure may cause cerebrovascular injury and the neurological complications of eclampsia (33).

Despite the large number of studies regarding AQP4 expression and function in the brain, little is known about the physiological role of this protein in human placenta. The placenta is a tissue where flow regulation from mother to fetus is crucially important in allowing normal fetal development and growth (34). In this study, we investigated AQP4 expression in human placenta during pregnancy. We observed, in the first trimester of gestation, an intense level of AQP4 expression in syncytiotrophoblast, the outer layer of placental villous together with a moderate level of expression in the cytoplasm of endothelial cells inside placental villi. During the third trimester of gestation, we observed a decrease of AQP4 expression in the syncytiotrophoblast in contrast with an increase of AQP4 immunopositivity in the cytoplasm of endothelial cells. The

AQP4 expression pattern in human placenta during gestation seems to suggest an important role of this protein in the regulation of maternal-fetal fluid exchange between placental cells and capillaries. In addition, AQP4 could play a key role in the regulation of amniotic fluid volume homeostasis. In conclusion, since there is a requirement for increased fluid transfer to the conceptus, the expression of AQP4 in human placenta throughout pregnancy seems to be important to maintain fetal growth.

References

- King LS and Agre P: Pathophysiology of the aquaporin water channels. *Annu Rev Physiol* 58: 619-648, 1996.
- Verkman AS and Mitra AK: Structure and function of aquaporin water channels. *Am J Physiol* 278: F13-F28, 2000.
- Papadopoulos MC, Krishna S and Verkman AS: Aquaporin water channels and brain edema. *Mount Sinai J Medicine* 69: 242-248, 2002.
- Ishibashi K, Kuwahara M and Sasaki S: Molecular biology of aquaporins. *Rev Physiol Biochem Pharmacol* 141: 1-32, 2000.
- Agre P, Bonhivers M and Borgnia MJ: The aquaporins, blueprints for cellular plumbing systems. *J Biol Chem* 273: 14659-14662, 1998.
- Verkman AS: Aquaporin water channels and endothelial cell function. *J Anat* 200: 617-627, 2002.
- Amiry-Moghaddam M and Ottersen OP: The molecular basis of water transport in the brain. *Nature Rev Neurosci* 4: 991-1001, 2003.
- Quick A and Cipolla MJ: Pregnancy-induced up-regulation of aquaporin-4 protein in brain and its role in eclampsia. *FASEB J* 19: 170-175, 2005.
- Silberstein C, Bouley R, Huang Y, Fang P, Pastor-Soler N, Brown D and Van Hoek AN: Membrane organization and function of M1 and M23 isoforms of aquaporin-4 in epithelial cells. *Am J Physiol Renal Physiol* 287: F501-F511, 2004.
- Hasegawa H, Ma T, Skach W, Matthay MA and Verkman AS: Molecular cloning of a mercurial-insensitive water channel expressed in selected water-transporting tissues. *J Biol Chem* 269: 5497-5500, 1994.
- Jung JS, Bhat RV, Preston GM, Guggino WB, Baraban JM and Agre P: Molecular characterization of an aquaporin cDNA from brain: candidate osmoreceptor and regulator of water balance. *Proc Natl Acad Sci USA* 91: 13052-13056, 1994.
- Yang B, Ma T and Verkman AS: cDNA cloning, gene organisation and chromosomal localization of a human mercurial insensitive water channel. *J Biol Chem* 270: 22907-22913, 1995.
- Ma T, Yang B and Verkman AS: Gene structure, cDNA cloning, and expression of a mouse mercurial insensitive water channel. *Genomics* 33: 382-388, 1996.
- Nielsen S, Nagelhus EA, Amiry-Moghaddam M, Bourque C, Agre P and Ottersen OP: Specialized membrane domains for water transport in glial cells: High resolution immunogold cytochemistry of aquaporin-4 in rat brain. *J. Neurosci* 17: 171-180, 1997.
- Rash JE, Yasumura T, Hudson CS, Agre P and Nielsen S: Direct immunogold labeling of aquaporin-4 in square arrays of astrocyte and ependymocyte plasma membranes in rat brain and spinal cord. *Proc Natl Acad Sci USA* 20: 11981-11986, 1998.

- 16 Papadopoulos MC, Manley GT, Krishna S and Verkman AS: Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. *FASEB J* 18: 1291-1293, 2004.
- 17 Amiry-Moghaddam M, Xue R, Haug F-M, Neely JD, Bhardwaj A, Agre P, Adams ME, Froehner SC, Mori S and Ottersen OP: Alpha syntrophin deletion removes the perivascular but not the endothelial pool of aquaporin-4 at the blood-brain barrier and delays the development of brain edema in an experimental model of acute hyponatremia. *FASEB J* 18: 542-544, 2004.
- 18 Verkman AS, Yang B, Song Y, Manley GT and Ma T: Role of water channels in fluid transport studied by phenotype analysis of aquaporin knockout mice. *Exp Physiol* 85: 233S-241S, 2000.
- 19 Verkman AS: Lessons from renal phenotype of aquaporin null mice. *Curr Opin Nephrol Hypertens* 9: 517-522, 2001.
- 20 Li J and Verkman AS: Impaired hearing in mice lacking aquaporin-4 water channels. *J Biol Chem* 276: 31233-31237, 2001.
- 21 Yang B, Verbavatz JM, Song Y, Vetrivel L, Manley G, Kao WM, Ma T and Verkman AS: Skeletal muscle function and water transport in aquaporin-4 deficient mice. *Am J Physiol* 278: C1008-C1115, 2000.
- 22 Wang KS, Komar AR, Ma T, Filiz F, McLeroy J, Hoda K, Verkman AS and Bastidas JA: Gastric acid secretion in aquaporin-4 knockout mice. *Am J Physiol* 279: G448-G453, 2000.
- 23 Richard C, Gao J, Brown N and Reese J: Aquaporin water channel genes are differentially expressed and regulated by ovarian steroids during the periimplantation period in the mouse. *Endocrinology* 144: 1533-1541, 2003.
- 24 Ohara M, Martin P-Y, Xu D-L, St. John J, Pattison TA, Kim JK and Schrier RW: Upregulation of aquaporin 2 water channel expression in pregnant rats. *J Clin Invest* 101: 1076-1083, 1998.
- 25 Butlin D: The Placenta – a literature review. Online at www.simba.rdg.ac.uk
- 26 Cobellis L, De Falco M, Mastrogiacomo A, Giraldi D, Dattilo D, Scaffa C, Colacurci N and De Luca A: Modulation of apelin and APJ receptor in normal and preeclampsia-complicated placentas. *Histol Histopathol* 22: 1-8, 2007.
- 27 Seelam B, Kayisli UA, Mulayim N and Arici A: Regulation of Fas ligand expression by estradiol and progesterone in human endometrium. *Biol Reprod* 65: 979-985, 2001.
- 28 Saadoun S, Papadopoulos MC, Davies DC, Krishna S and Bell BA: Oedematous human brain tumours have increased aquaporin-4 expression. *J Neurol Neurosurg Psychiatry* 72: 262-265, 2002.
- 29 Frigeri A, Gropper MA, Turck CW and Verkman AS: Immunolocalization of the mercurial-insensitive water channel and glycerol intrinsic protein in epithelial cell plasma membranes. *Proc Natl Acad Sci USA* 92: 4328-4331, 1995.
- 30 Frigeri A, Gropper MA, Umenishi F, Kawashima M, Brown D and Verkman AS: Localization of MIWC and GLIP water channel homologs in neuromuscular, epithelial and glandular tissues. *J Cell Sci* 108: 2993-3002, 1995.
- 31 Yang B and Verkman AS: Water and glycerol permeability of aquaporins 1-5 and MIP determined quantitatively by expression of epitope-tagged constructs. *J Biol Chem* 272: 20782-20786, 1997.
- 32 Amiry-Moghaddam M, Otuska T, Hurn PD, Traystman RJ, Haug F-M, Froehner SC, Adams ME, Neely JD, Agre P, Ottersen OP and Bhardwaj A: An alpha-syntrophin dependent pool of AQP4 in astroglial end-feet confers bidirectional water flow between blood and brain. *Proc Natl Acad Sci USA* 100: 2106-2111, 2003.
- 33 Belfort MA, Varner MW, Dizon-Townson DS, Grunewald C and Nisell H: Cerebral perfusion pressure, and not cerebral blood flow, may be the critical determinant of intracranial injury in preeclampsia: a new hypothesis. *Am J Obstet Gynecol* 187: 626-634, 2002.
- 34 Liu H, Koukoulas I, Ross MC, Wang S and Wintour EM: Quantitative comparison of placental expression of three aquaporin genes. *Placenta* 25: 475-478, 2004.

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