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# CFTR May Modulate AQP9 Functionality in Preeclamptic Placentas

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ABSTRACT

Preeclampsia (PE) is a hypertensive disorder unique to human pregnancy. Although its causes remain unclear, it is known that altered placental villous angiogenesis and a poorly developed fetoplacental vasculature can affect the transport functions of the syncytiotrophoblast (hST).

We have previously observed that in preeclamptic placentas there is an increase in AQP9 protein expression, with a lack of functionality. Up to now, the mechanisms for AQP9 regulation and the role of AQP9 in the human placenta remain unknown. However, there is strong evidence that the cystic fibrosis transmembrane conductance regulator (CFTR) regulates AQP9 functionality.

*Objective:* Here, we studied CFTR expression and localization in hST from preeclamptic placentas in order to investigate if alterations in CFTR may be associated with the lack of activity of AQP9 observed in PE.

*Methods:* The expression of CFTR in normal and preeclamptic placentas was determined by Western Blot and immunohistochemistry, and CFTR-AQP9 co-localization was determined by immunoflurescence. Water uptake experiments were performed using explants from human normal term and preeclamptic placentas treated with CFTR inhibitors.

*Results:* We found that CFTR expression significantly decreased in preeclamptic placentas, and that the hST apical labeling almost disappeared, losing its co-localization with AQP9. Functional experiments demonstrated that water uptake diminished in normal term explants incubated with CFTR inhibitors.

*Conclusions:* These results suggest that CFTR expression decreases in preeclampsia and may thus be implicated in the regulation of AQP9 activity.



## 1. Introduction

Nutrients, antibodies, infectious agents and other substances passing from maternal to fetal circulation within the villous placenta have to cross the continuous, mitotically inactive, multinucleated syncytiotrophoblast (hST) cells. This tissue that results from the fusion of the underlying cytotrophoblast cells forms a syncytium with minimal tight junctions. Consequently, the transport from mother to fetus should take place primarily via transcellular routes [1–3]. Nevertheless, physiological data indicate that both a transcellular and a paracellular pathway are available for transfer across the human placenta, but the morphological correlate of the latter is uncertain [4] and the possibility exists that wide, non-specific, paracellular channels, allowing the passage of large hydrophilic molecules, may also be present [5,6]. However, the molecular mechanisms of these processes are little known.

Transcellular water flux may be facilitated by aquaporins (AQPs). AQPs are a family of small integral membrane proteins (30 kDa monomers) that transport either water alone or water and small solute(s) such as glycerol. AQPs increase cell plasma membrane water permeability 5- to 50-fold as compared with membranes where water moves primarily through the lipid bilayer [7–9].

There are at least 13 AQPs in mammals which show a wide range of distribution in organs that are actively involved in water movement. According to their structural and functional properties, AQPs are divided into two subgroups: "classical aquaporins" (AQP0, 1, 2, 4, 5, 6 and 8, selective for water), and "aquaglyceroporins" (AQP3, 7, 9 and 10, permeable to both water and neutral solutes). AQP11 and AQP12 have been recently identified and are more distantly related [10,11].

Aquaporin 9 (AQP9) is a member of the aquaglyceroporin subfamily of AQPs and shares the highest amino acid sequence homology with AQP3, AQP7, and AQP10 [12,13]. In addition to water, AQP9 transports small uncharged molecules like glycerol,

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urea, purines, and pyrimidines [14,15], but its physiological function(s) remains unknown. AQP9 expression has been reported in many tissues [16]. We have previously reported the expression of aquaglyceroporins permselective to urea and glycerol (AQP3) as well as to a broad range of small solutes (AQP9) in hST from normal human placenta [17]. We proposed that these aquaglyceroporins may participate not only in the water transport between mother and fetus but may also play a role in the rapid movement of solutes across cell membranes with minimal osmotic perturbation. Recently, we have described an increase in AQP9 expression in hST from preeclamptic placentas with a lack of functionality for water and mannitol [18].

Little is known about AQP9 regulation. However, the presence of numerous sites of regulation in the gene and on the protein has been described.

Studies in liver and brain suggested that a negative insulin response element (IRE) in the promoter region participates in this regulation of levels of AQP9 protein [19,20], Tsukaguchi et al. also described a putative hypertonicity response element in the promoter region of AQP9 [14]. In addition, several pathways leading to regulation of AQP9 expression have been identified including protein kinases A (PKA) and C (PKC) [21,22]. Despite the presence of consensus sites for phosphorylation by PKC on the AQP9 protein, direct regulation of the channel by phosphorylation has not yet been observed [22].

Mitogen activated protein kinase (MAP-kinase) pathways, P38 MAP-kinase, were also shown to be implicated in an increase of AQP9 expression after an osmotic stress and ischemic infarct [23,24].

However, up to now, the functional regulation of AQP9 has not been clarified.

Several previous reports indicate that cystic fibrosis transmembrane conductance regulator (CFTR) is able to interact with various membrane proteins by regulating their transport activity as well as by functioning as a cAMP-regulated chloride channel [25]. Among them are the epithelial $Na^+$ channels [26,27] and the outwardly rectifying $Cl^-$ channels [28]. CFTR is also involved in the regulation of water flux by AQPs [29–31]. So far, there is no evidence that CFTR could affect their molecular expression. This channel provides an exit pathway for secondary active chloride transport from blood to lumen, followed passively by sodium. The resulting accumulation of NaCl in the lumen generates an osmotic gradient for water secretion via AQPs.

Cheung et al. reported an interaction between epididymal CFTR and AQP9 and found a significant increase in water permeability, consistent with a synergistic effect of the two proteins in conferring water permeability in *Xenopus* oocytes. Therefore, they concluded that CFTR activation is required for AQP9 to increase its activity [30].

CFTR has been found on the apical membrane of hST from human normal term placenta [32] and serves as a conductive pathway for anions, whereas AQPs are related with water transport. These proteins are therefore essential for normal fetal growth and development.

In this study, we focused on the hypothesis that, in human placenta, CFTR interacts with AQP9, modulating its activity. Hence, the aim of our study was to establish whether the AQP9 functionality correlates with the molecular expression of CFTR in normal and preeclamptic placentas.

## 2. Materials and methods

### 2.1. Tissue collection

This study was approved by the local ethics committees of the "Hospital Nacional Dr. Prof. Alejandro Posadas", Buenos Aires, Argentina, and written consent was obtained from patients before the collection of samples.

**Table 1**
Patient characteristics (mean ± SEM).

| | Term control | Severe preeclampsia |
|---|---|---|
| Number of pregnant women | 6 | 6 |
| Maternal age, year | 23.6 ± 1.3 | 26.4 ± 2.1 |
| Gestational age, week | 39.7 ± 0.8 | 37.1 ± 0.4 |
| Mean blood pressure, mmHg | | |
| Systolic | 110 ± 4.2* | 160.5 ± 4.5* |
| Diastolic | 67 ± 3.5** | 110.0 ± 2.8** |
| Birth weight, g | 3070 ± 250 | 2670 ± 294 |

*$P < 0.01$; **$P < 0.01$.

Full-term normal and preeclamptic placental tissues were obtained after cesarean section. Normal pregnant patients ($n = 6$) had maternal blood pressures ≤ 110/70 mmHg, no proteinuria, and no other complications. Preeclamptic patients ($n = 6$) had gestational hypertension > 140/90 mmHg, with proteinuria that developed for the first time during pregnancy. The gestational age was 37–42 weeks and the maternal age had a range between 20–30 years old in both groups of patients. No differences were observed between the newborn weight from normal and preeclamptic mothers. (Table 1)

### 2.2. Immunoblotting

Human placenta villi from normal term and preeclamptic placentas were processed according to the method previously described [17]. Briefly, human chorionic villi were fragmented, and washed with unbuffered 150 mM NaCl. The tissue was then shaken for 1 h with 1.5 volumes of HES buffer (10 mM HEPES-KOH, 0.1 mM EGTA, 250 mM sucrose) pH 7.4, with protease inhibitors (0.2 mM PMSF, 25 μg/mL *p*-aminobenzamidine, 20 μg/mL aprotinin, 10 μg/mL leupeptin, 10 μg/mL pepstatin), followed by filtration and centrifugation at 3100 *g* for 10 min. The supernatant was then further centrifuged for 10 min at 11,000 *g* and the resulting supernatant centrifuged for 70 min at 16,000 *g*.

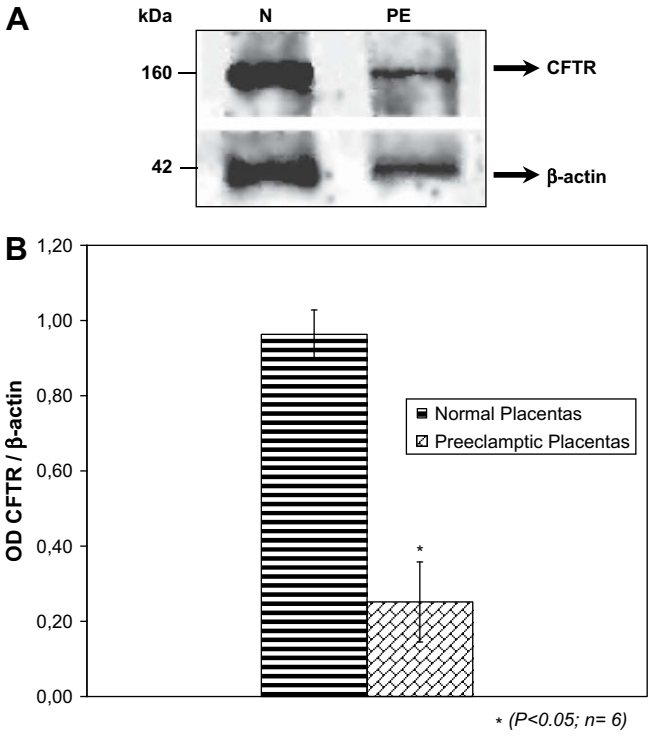


**Fig. 1.** Semiquantitative immunoblotting analysis of CFTR abundance in hST. (A) A representative immunoblot shows that CFTR protein level expression was weakly detectable in preeclamptic placentas (PE) as compared to normal ones (N). β-actin expression was determined to control for unequal loading. (B) Densitometry of immunoblots containing AQP9 protein level expression was performed, and after normalization for β-actin, the values were plotted as the AQP9/β-actin relative ratio. Each plotted value corresponds to the mean ± SEM obtained from six placentas. *$P < 0.05$.

The pellet was then resuspended in HES buffer containing 10 mM $MgCl_2$ to selectively precipitate non-apical membranes. The suspension was then incubated 10 min with constant stirring, after which it was centrifuged for 30 min at 5000*g*. The basal membrane-enriched pellet was redissolved in HES buffer containing protease inhibitors, and stored at −80 °C. Finally, the supernatant was centrifuged for 70 min at 16,000 *g* and the apical membrane-enriched pellet was resuspended in HES buffer containing proteases inhibitors, and stored at −80 °C until assayed for biochemical markers. Alkaline phosphatase activity (an apical plasma membrane enzymatic marker) in the final membrane suspension was >20, while ratios for those enzymes, which are markers of non-apical membranes, were <1.5 [17].

For immunoblotting studies, 10–20 μg of membrane fraction proteins was dissolved in loading buffer (4% sodium dodecyl sulphate, 0.125 M Tris–HCl pH 6.8, 0.2 M dithiothreitol, 0.02% bromophenol blue, 20% glycerol), heated to 90 °C for 2 min, resolved on 15% polyacrylamide gel and electrotransferred onto nitrocellulose membranes (Hybond ECL, Amersham Pharmacia Biotech Ltd., UK). Membranes were blocked with 1 (w/v) bovine serum albumin in TBS at room temperature for 30 min and incubated overnight with a mouse monoclonal primary antibody (R&D Systems, USA) against the R domain (MAb-13-1) of human CFTR diluted 1:1000. Membranes were washed with TBS–Tween 0.1%, and incubated at room temperature for 2 h with a goat anti-mouse IgG (Sigma) (1:1000) conjugated to alkaline phosphatase. Filters were washed and incubated with the substrate BCIP/NBT (5-bromo-4chloro-3-indolyl phosphate/nitro blue tetrazolium; Promega).

To confirm equal loading, each membrane was stripped and analyzed for β-actin protein expression, demonstrating that the band intensities did not present significant changes between the samples studied. Briefly, membranes were incubated overnight with monoclonal anti-β-actin (Alpha Diagnostic International Inc., USA) diluted 1:2000. After washing, membranes were incubated at room temperature for 2 h with a goat anti-mouse IgG (Sigma) (1:1000) conjugated to alkaline phosphatase. Then, they were revealed as we have described and the immunoreactive signals estimated by densitometry.

### 2.3. Immunohistochemistry

#### 2.3.1. Immunoperoxidase

Human villous tissue from normal and preeclamptic term placentas were cut into small pieces, fixed overnight in 10% formaldehyde–0.1 M sodium phosphate buffer (PBS), pH 7.4, dehydrated, and embedded in paraffin. Paraffin sections (4 μm) were cut and mounted on 2% silanized slices, dried, dewaxed and rehydrated. Tissue slices were incubated in 3% $H_2O_2$/methanol for 5 min to block endogenous peroxidase, and washed three times in 10 mM PBS, pH 7.4, for 5 min. All subsequent steps were carried out in a humidified chamber. Non-specific binding sites were blocked by incubation at room temperature in blocking reagent (DAKO LSAB kit, Dako Corp., USA) for 30 min. Sections were incubated overnight with CFTR or AQP9 antibody (Alpha Diagnostic, USA) at a dilution of 1:50 or 1:100 respectively. Sections were then washed with 0.05 M PBS for 5 min, incubated for 10 min in prediluted link antibody, washed again in PBS buffer and incubated for 30 min in a solution of streptavidin-conjugated horse-radish peroxidase in PBS. Color development of the antibody labeling was achieved under microscopic control by incubating slices with the substrate DAB/$NiCl_2$ (3,3′-diaminobenzidine/nickel chloride) and 0.3% hydrogen peroxide in distilled water. Counterstain by hematoxylin was performed. Non-immune mouse serum without primary antibody was used as a negative control.

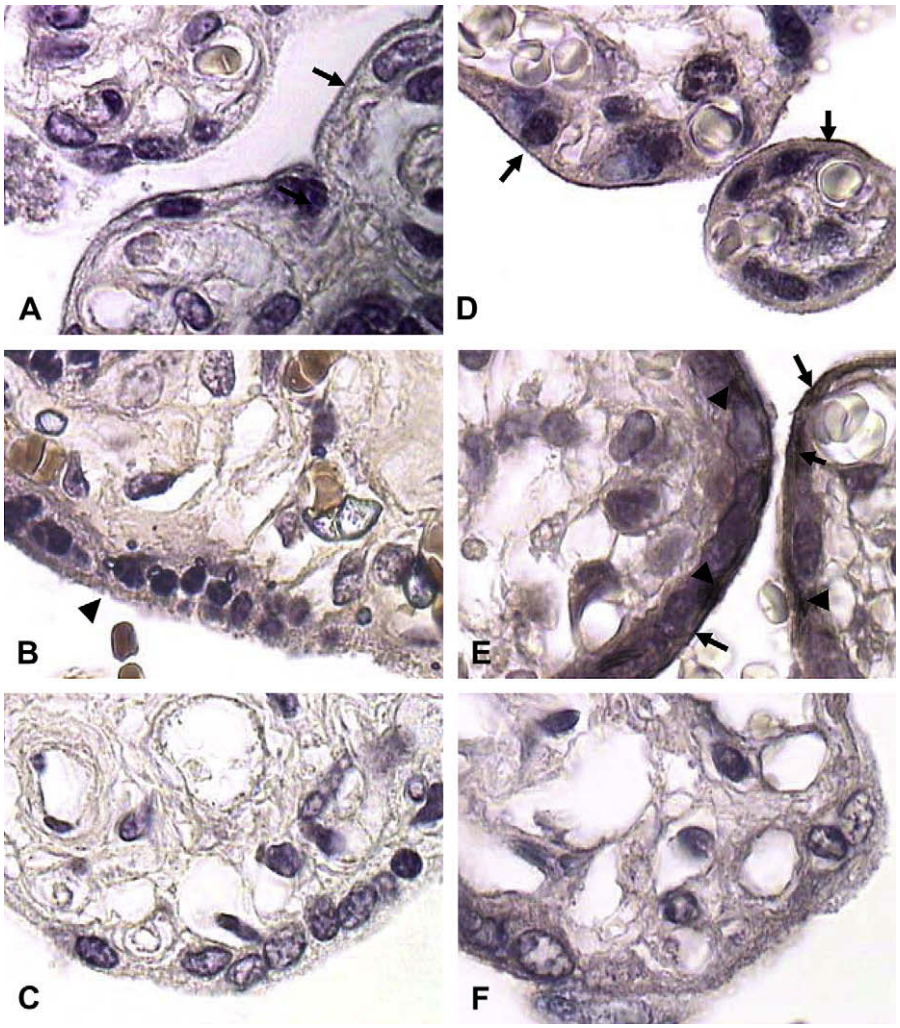


**Fig. 2.** Localization of CFTR (A–C) and AQP9 (D–E) proteins in hST from normal and preeclamptic placentas. Immunostaining with an anti-CFTR antibody revealed specific labeling in the apical membrane of hST from normal placentas (arrows in A). In preeclamptic placentas, CFTR immunolabeling was almost undetectable in apical membranes (arrowhead in B). AQP9 was located in the apical membrane of hST from normal placentas (arrows in D), while in preeclamptic placenta AQP9 was located in the apical, basal membranes (arrows in E), and cytoplasmic regions (arrowhead in E). Negative controls were performed by omitting the primary antibody and replaced by a non-immune rabbit serum (C and F). Magnification: 1000×.

*2.3.2. Double immunofluorescence*

For Immunofluorescence experiments, the slides were preincubated with non-immune rabbit serum in PBS (1:100) in a wet chamber at room temperature for 30 min, following the incubation with a mix containing the monoclonal anti-CFTR antibody (1:40 PBS) and the polyclonal anti-AQP9 antibody (1:100 in PBS) overnight in a wet chamber at 4 °C. After several rinses in PBS, the slides were incubated with a mix of secondary anti-rabbit IgG antibody conjugated with Cy3 (Jackson, USA) (dilution 1:800) together with an anti-mouse IgG antibody conjugated with fluorescein (dilution 1:400) (Santa Cruz Biotechnology, USA) both diluted in PBS and incubated for 2 h at room temperature in a wet chamber. Finally, all the slides were rinsed in PBS, mounted on a mixture containing PBS:glycerol (1:3), and observed in a double labeling epifluorescent microscope (Nikon, Eclipse E200) provided with green and red filters. Negative controls were performed using CFTR and AQP9 antibodies preadsorbed with the specific peptides.

*2.4. Tissue culture*

Within 1 h of delivery, samples of chorionic villi from human normal term and preeclamptic placental cotyledons were dissected. Placental tissue was gently separated by sterile dissection from different cotyledons, excluding chorionic and basal plates, minced with scalpel blades, and washed repeatedly with 0.9% sodium chloride to remove blood from the intervillous space. Whole villous tissue (~50 mg/well) was incubated in 24-well polystyrene tissue culture dishes in 2 mL of serum-free Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Inc. BLR, Grand

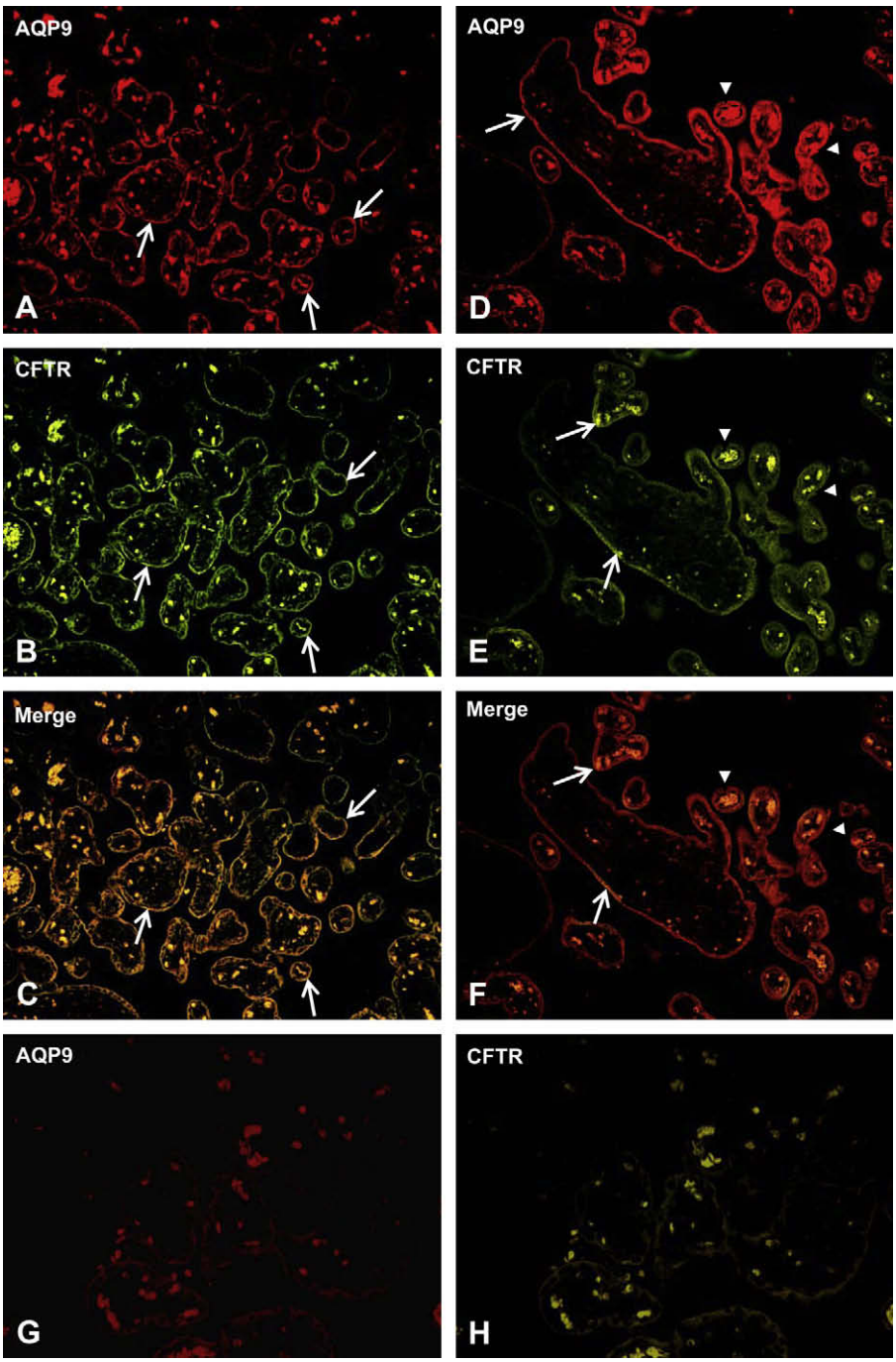


**Fig. 3.** Double immunofluorescence labeling of CFTR and AQP9 proteins. Normal placenta (A–C). AQP9 was also observed in apical membrane (A; white arrow). CFTR was localized in the apical membrane of hST (B; white arrow), co-localization CFTR/AQP9 in apical membrane of hST (C; merge, white arrow). Preeclamptic placenta (D–F). AQP9 signal was increased (D; white arrowhead). Areas with normal expression (E; white arrowhead) and decreased expression of CFTR (D; white arrowhead) were observed. Co-localization CFTR/AQP9 was decreased (F; merge, white arrowhead). Negative controls (G and H). Magnification: 200×.

Island, N.Y.) containing 100 IU/mL penicillin, 100 mg/mL streptomycin, 32 mg/mL gentamycin at 37 °C for 24 h in a humidified gas mixture of 5% $CO_2$ and 95% air [18]. Supernatants were collected and stored at −20 °C for hCG analysis. The cultures were kept for up to seven days for functional and morphological evaluation, respectively. Cultures with apparent bacterial contamination were interrupted and excluded.

### 2.5. Histological studies

Explants from normal and preeclamptic placental villi were histologically evaluated at different incubation times ranging from 0 to 48 h. The tissues were harvested and fixed in 10% formaldehyde–0.1 M PBS pH 7.4, dehydrated, and embedded in paraffin, until further processing. The pieces were dehydrated in an ascending alcohol series. Sections (0.5 μm thick) were cut, mounted on glass slides, and stained with Hematoxylin and Eosin and observed at light microscopy.

### 2.6. Analysis of collected culture medium

Explant viability was determined by the production of β-human chorionic gonadotrophin. The concentration of β-hCG in the culture medium was assessed by quantitative immunoradiometric determination (IRMA) using a commercially available kit (hCG solid phase component system, Coat-A-Coat hCG IRMA, EURO/DPC Ltd., UK). The hCG assay uses the "sandwich technique", where the solid phase binds the alpha subunit of hCG and a radiolabeled antibody in the liquid phase binds to the beta one.

### 2.7. Water uptake

Experiments were undertaken using placentas from normal term and preeclamptic pregnancies. Placental explants were incubated in duplicate at room temperature, in 0.5 mL Ringer's solution containing $^3$HOH [New England Nuclear Corp]. The uptake was stopped by adding ice-cold Ringer solution. At the end of the incubations, explants were quickly washed in cold Ringer's solution. Further, each explant was placed in a polystyrene vial, solubilized with 1.0 mL of 1 M sodium hydroxide overnight at 37 °C. Aliquots of the solubilized explants were vortex-mixed with 2.0 mL of scintillant (Optiphase "HiSafe", Wallc Oy, England) and counted on a scintillation counter. Other aliquots were kept for determination of protein concentration by the BCA assay (Pierce, Rockford, IL, USA) according to the manufacturer's protocol using bovine serum albumin as a standard.

Inhibition studies with 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS), glibenclamide, and diphenylamine-2-carboxylate (DPC) were carried out in placental normal and preeclamptic explants previously incubated for 10 min in Ringer solution with 500 μM DIDS, 500 μM DPC or 80 μM glibenclamide. Solute uptake experiments were then performed as described above.

### 2.8. Statistical analysis

Average data values are expressed as mean ± SEM of solute picomoles (pmol) per milligram (mg) of protein per minute (min). $n$ is the number of placentas tested under each condition. The uptake data ($pmol \cdot mg^{-1} \cdot min^{-1}$) obtained from each group were compared by one-way analysis of variance (ANOVA test) followed by Fisher LSD test. Normal distribution was assessed by Kolmogorov–Smirnov test. All statistical analysis was carried out with the *Statistica software version 6.0*. The criterion for statistical significance was $P < 0.05$.

## 3. Results

### 3.1. Reduced abundance of CFTR protein in preeclamptic placenta

Membrane fraction proteins from normal and preeclamptic term placentas were probed with an anti-CFTR antibody and quantified using β-actin as an internal standard. In normal placentas the antibody revealed an expected band of 160 kDa. This band was weakly detected in preeclamptic placentas (Fig. 1). Semiquantitative analysis showed that CFTR decreased 3.8-fold in preeclamptic placentas.

No bands were observed when the primary antibody was omitted or when the CFTR antibody was preadsorbed with the CFTR specific peptide (data not shown).

Immunohistochemistry studies in normal placentas showed the localization of CFTR and AQP9 in the apical membrane of hST. In both cases, immunolabeling was not observed in cytotrophoblast stroma and endothelial cells. In preeclamptic placentas, CFTR was strongly decreased, in agreement with the immunoblotting assay (Fig. 2), while AQP9 was located not only in the apical and basal membranes of hST, but it was also distributed in the cytoplasmic region. Control sections treated with non-immune serum showed absence of labeling.

### 3.2. Co-localization of CFTR with AQP9

Double immunofluorescence labeling revealed expression of CFTR protein in the apical membrane of hST co-localized with AQP9 in normal placentas. In the apical membrane of hST from preeclamptic placentas, CFTR was weakly detected, but AQP9 showed an increase in apical, basal and intracellular staining (Fig. 3).

### 3.3. Explant viability

Explant viability from normal and preeclamptic placentas was examined at different times of incubation. The surface of trophoblast epithelial cells was not adversely affected by the experimental procedure until 24 h. The biochemical viability of the placental villi during incubation was assessed by estimating β-hCG concentrations in the extracellular medium.

All explants from normal and preeclamptic placentas produced β-hCG at the usual levels ($4.8 \pm 0.2$ IU/g, $n = 6$; $15.3 \pm 0.5$ IU/g, $n = 6$; respectively).

### 3.4. Water uptake

After having established the cellular viability of the placentas, we determined water uptake in order to analyze whether CFTR may modulate water permeability. Villous fragments were isolated from normal term placentas ($n = 6$) and were treated with diphenylamine-2-carboxylate (DPC), 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid (DIDs) (inhibitors of chloride channels) and glibenclamide (an open-channel blocker of CFTR). Water uptake was significantly reduced by all the inhibitors tested (DIDs and DPC ~35%, glibenclamide ~50% ($n = 6$, $P < 0.05$)) (Fig. 4). As we previously reported [18], water uptake in explants from preeclamptic placentas showed a decrease of ~51% ($n = 6$, $P < 0.05$) compared to normal placentas and it was not modified by the inhibitors tested.

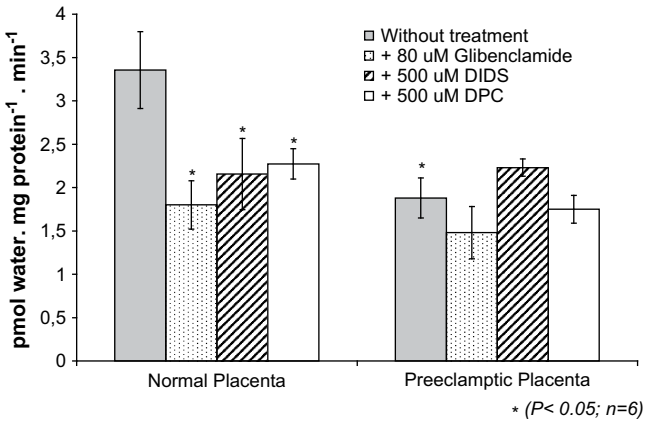


**Fig. 4.** Water uptakes in explants from normal term and preeclamptic placentas treated with CFTR inhibitors. In all cases a decrease in water uptake was observed. In normal placentas, treatment with 80 μM glibenclamide reduced water uptake by 47% while incubation with 500 μM DIDs or 500 μM DPC reduced it by 35% ($P < 0.05$, $n = 6$). However, water uptake in explants from preeclamptic placentas without treatment, showed a decrease of ~51% compared to normal placentas ($P < 0.05$, $n = 6$) and it was not significantly modified by the inhibitors tested. The data are shown as mean ± SEM.

## 4. Conclusions

The hST plays an important role throughout pregnancy because it produces most of the placental hormones that are required for normal fetal growth and development. The syncytial nature of this epithelium suggests that transport from mother to fetus should take place primarily via transcellular routes. Thus, transcellular water flux across human placenta may be facilitated by aquaporins.

Although we have previously found an increase in AQP9 protein localized in the plasma membrane of hST from preeclamptic placentas, AQP9 was not functional for water transport [18].

In this study, we investigated if the lack of AQP9 activity in preeclampsia is associated with alterations in the CFTR expression.

Both CFTR and AQP9 are localized on the apical membrane of the hST and they are supposed to play a role in electrolyte and water transport from mother to fetus [17,32]. Evidence in other epithelial tissues indicates that, besides functioning as a cAMP-activated chloride channel, CFTR also acts as a regulator of other membrane transport proteins such as AQP9 [30,31]. Previous studies have postulated that CFTR may be involved in the regulation of osmotic water flow and could play a key role in the regulation of AQP9. Cheung et al. have shown that CFTR confers a cAMP-dependent activation of AQP9 in *Xenopus* oocytes and that inhibition of CFTR reverses the cAMP activation of AQP9 in the intact cauda epididymis [30].

In the present study, we showed that CFTR protein expression decreased dramatically (3.8-fold) in the hST from preeclamptic placentas. Moreover, in normal placentas, CFTR co-localized with AQP9 in the apical membrane of the hST. However, in preeclamptic placentas where AQP9 is over-expressed, CFTR was almost undetectable. This reduced expression of CFTR in the plasma membrane may be implicated in the AQP9 functional decrease that we have previously reported [18].

As we previously reported [18], in explants from preeclamptic placentas water permeability decreased significantly compared to normal placentas and it was not modified by the inhibitors of CFTR. Here, we also observed a decrease in water permeability in explants from normal placentas treated with inhibitors tested.

Taken into account that there is no evidence that CFTR could function as a water channel per se, we suggest that the CFTR protein is required to preserve the normal functionality of AQP9. This finding is consistent with a synergist effect of the two proteins, and alterations in their expression may affect transcellular water transport.

The inductor mechanisms that cause CFTR protein decrease in preeclamptic placentas are not clear yet. Recently, we have reported that the apical membrane of preeclamptic hST is more rigid than normal hST, where sphingomyelin levels are 1.5-fold up, and where cholesterol levels are unchanged [33]. In addition, emerging evidences indicate that changes in membrane lipid composition may affect membrane order, fluidity and lipid–protein interaction [3,34–36]. Taken together, our results suggest that these changes in the lipid membrane composition probably contribute to create an unfavorable environment for CFTR insertion in the plasma membrane.

In conclusion, our results show for the first time, that CFTR protein expression is significantly reduced in preeclamptic placentas. We propose that the decrease in CFTR protein, possibly due to changes in the lipid composition of the syncytial membrane, may be affecting AQP9 functionality. We suggest that the potential effect of CFTR in the regulation of the function of AQP9 could be an important factor involved in the water transport equilibrium in the human placenta.

Further studies are needed to define whether the lack of CFTR, the increase in AQP9 expression and the concomitant functional alterations could be related with an adaptive response or could be involved as a direct pathogenic factor in the development of preeclampsia.

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