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Expression of aquaporins early in human pregnancy

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ABSTRACT

Background: Aquaporins (AQPs) constitute a family of channel proteins implicated in transmembrane water transport. Thirteen different AQPs (AQP0–12) have been described but their precise biologic function still remains unclear. AQPs 1, 3, 4, 8, and 9 expression has been described in human chorion, amnion and placenta; however, AQP4 is the only that has been identified in the first trimester of human pregnancy.
Objective: To assess multiplicity of AQPs expression from 10th to 14th week gestation.
Population and methods: Chorionic villi samples (CVS) collected in pregnant women for prenatal diagnosis were analysed by real time-PCR to assess cDNA expression of AQPs 1, 2, 3, 4, 5, 6, 7, 8, 9, and 11, and compared with AQPs expression in placentas from normal term pregnancies.
Results: 26 CVS corresponding to 26 pregnant women (age: 32.7 ± 4.5 years; gestational age: 12.4 ± 0.9 weeks) and 10 placental samples corresponding to normal term pregnancies were analysed. In CVS karyotype was normal in 16 cases, trisomy in 6 cases, mosaicism in 1 and unknown in 1. We found high mRNA expression for AQPs 1, 3, 9 and 11, low for AQPs 4, 5, and 8, and non-detectable for AQPs 2, 6, and 7 in chorionic villi.
Conclusions: This is the first study systematically assessing the expression of a multiplicity of AQPs in chorionic villi samples between 10th and 14th weeks of gestation. High expression of AQP11 has been identified for the first time in early stages of human pregnancy. Chromosomal abnormalities did not alter AQPs' expression.

1. Introduction

Water is an indispensable constituent of biological systems. Aquaporins, first discovered in 1991, constitute a family of small hydrophobic intra-membranous proteins (26–30 kDa) functioning as cell membrane water channels [1,2]. Of note, compared to the traditionally described transmembrane transfer across the lipid by-layer water permeability across AQP water channels may be increased up to 50 fold [3]. In addition, a specific subset of AQPs namely AQPs 3, 7, 9 and 10 referred to as "aquaglyceroporins" is also permeable to water, urea and glycerol; moreover, AQP9 also facilitates the flux of neutral solutes such as monocarboxylates, purines and pyrimidines [4]. To date, 13 members of the AQP family have been described in humans [5]. The role of AQPs in the regulation of placental water transfer to the foetus and intramembranous resorption is still only poorly understood; however, experimental and clinical data support the hypothesis that AQPs play an important role in foetal water flow (for review [4]). Foetal weight increases exponentially during gestation and foetal water requirements do accordingly [6]. Hence, at the end of gestation up to 400 mL/day are transferred from the amniotic cavity across the foetal membranes into the foetal circulation [7]. Interestingly, mechanisms regulating AF volume are yet not fully understood it has been suggested that aquaporins (AQPs) could play an important role in the regulatory process [5]. Remarkably, abnormal placental transfer of fluid may result in excessive (polyhydramnios) or reduced (oligohydramnios) amniotic fluid volume putting the foetus at risk of significant morbidity [8,9]. Moreover, increased AQP9 expression, protein synthesis and functionality have been associated with preeclamptic placentas modulated by human chorionic gonadotropin via cAMP pathways [10]. AQPs 1, 3, 4, 8 and 9 have been located in human placenta and foetal membranes late in gestation and consistently associated with the regulation of placental, chorion and amnion water transfer [4]. In this regard, several studies have correlated AQP9 and AQP8 over-expression with polyhydramnios and decreased AQP1 and AQP3 expression with

Abbreviations: AF, amniotic fluid; AQPs, aquaporins; CV, chorionic villi; CVS, chorionic villi sampling; RT-PCR, real time polymerase chain reaction; RNA, ribonucleic acid; mRNA, messenger ribonucleic acid; cDNA, complementary deoxy-ribonucleic acid; C_t, threshold cycle; NPA, asparagine–proline–alanine motif.

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oligohydramnios [11,12]. Notwithstanding, expression and role of AQPs early in human pregnancy when water transport to the foetus is still quantitatively not so relevant are lacking. Considering this, we hypothesized that AQPs expression towards the end of embryogenesis and initiation of organogenesis might be different than in later stages of pregnancy and could contribute to explain AQPs physiologic and pathophysiologic implications.

The aim of this study was to analyse the expression of AQPs in chorionic villi sampled between 10th and 14th week gestation using real time polymerase chain reaction (RT-PCR) and compare it to placental expression in term pregnancies. We omitted the determination of AQPs 0, 10 and 12 because they are specifically and uniquely expressed in the eye, gastrointestinal tract and pancreas respectively [5,13,14].

2. Material and methods

2.1. Study design

This is a prospective clinical study performed in both the Division of Obstetrics and the Division of Neonatology of the University Hospital La Fe (UHLF; Valencia; Spain) between January 2006 and September 2008 approved by the Internal Board Review (Comité de Ética e Investigación Clínica) of the UHLF. All participating women received, understood, and signed an informed consent prior to sample collection. It should be underscored that chorionic villi sampling (CVS) was not performed exclusively to determine expression of AQPs but following medical indications. All participants accepted that a minimal quantity of the tissue sampled was used for research purposes.

The inclusion criteria for the selection of the patients were: (i) spontaneous pregnancy; (ii) single pregnancy; (iii) pregnancy strictly controlled following routines of the Division of Obstetrics (UHLF). Exclusion criteria were: (i) co-morbidity or medication susceptible of influencing foetal fluid balance or AQP expression (e.g.: drugs interfering with renal function); (ii) multiple gestation; (iii) employment of assisted reproduction techniques. Patients' data and any other important co-morbidity were anonymously reported in a database.

CVS was performed trans-vaginally. Indications for CVS are shown in Table 1. The outcomes of the prenatal diagnostic and the pregnancy were processed in the database as well. Placental tissue samples (trophoblast) from 5 normal term pregnancies were used as controls.

2.2. Tissue collection and processing

Chorionic villi (CV) samples were soaked in RNA*later*® solution (Ambion, Carlsbad, California, USA) and kept at 4 °C overnight. Thereafter, supernatant was removed and samples were frozen at −80 °C. Placental (trophoblast) samples were collected from non-complicated term pregnancies (n = 5) and also soaked in RNA*later*® for a few hours, and then stored as for CV. Human samples of liver, uterus, and cerebral artery were obtained from the Division of Pathology of Hospital La Fe (Valencia, Spain). PANC-1 (cells of human carcinoma of the exocrine pancreas) and HEK293 (human embryonic kidney) cells were grown in DMEM 4.5 g/L glucose supplemented with 10% FBS, penicillin and streptomycin. After 24 h culture medium was removed and cells were re-suspended in TRIzol (Invitrogen Corporation, Carlsbad, CA, USA).

2.3. RNA extraction and cDNA synthesis

Total RNA was isolated using TRIzol (Invitrogen Corporation Carlsbad, CA, USA) reagent following the manufacturer's protocol. RNA concentration was quantified by spectrophotometry at 260 nm. Then, RNA was reverse transcribed to cDNA using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas Life Sciences, Canada) following manufacturer's instructions. All samples were treated (reverse transcribed and PCR amplified) simultaneously to avoid batch-to-batch bias.

Table 1
Summary of patients' characteristics and indications for chorionic villi sampling (CVS).

Case #	Maternal age (years)	CVS performed at g. age (weeks^{+days})	Indication for CVS	Karyotyping
1	36	10^{+6}	Nuchal translucency 6.8 mm	Trisomy 21
2	26	13^{+3}	Cystic hygroma	Normal
3	35	12^{+5}	Cystic hygroma	Normal
4	30	14^{+1}	Chromosomal abnormality in mother	Normal
5	38	13^{+0}	Previous son with mutation in NF1	Normal
6	28	12^{+0}	Previous son with X-linked recessive inheritance	Normal
7	31	12^{+4}	Nuchal translucency 6 mm	Trisomy 21
8	36	12^{+6}	Nuchal translucency 6.3 mm	Normal
9	26	12^{+3}	Cystic hygroma	Normal
10	37	11^{+3}	Father carrier of IT-15mutation	Normal
11	30	13^{+0}	Chromosomal abnormality in mother	Mosaicism
12	33	13^{+6}	Nuchal translucency 6.3 mm	Normal
13	38	12^{+5}	Cystic hygroma	Normal
14	37	13^{+3}	Nuchal translucency 3.8 mm	Mosaicism
15	38	12^{+4}	Foetus suspected hydrops	Normal
16	32	13^{+5}	Foetus suspected hydrops	Normal
17	31	11^{+4}	Nuchal translucency 5.5 mm	Normal
18	41	12^{+4}	Increased risk for Down's syndrome 1st trimester	Trisomy 21
19	37	11^{+6}	Nuchal translucency 6.8 mm	Trisomy 13
20	36	12^{+4}	Nuchal translucency 4.8 mm	Normal
21	29	12^{+0}	Nuchal translucency 6.7 mm	Normal
22	32	13^{+1}	Foetus suspected hydrops	Normal
23	39	12^{+4}	Nuchal translucency 7.3 mm	Trisomy 18
24	27	13^{+5}	Nuchal translucency 5.6 mm	Unknown
25	41	12^{+1}	Increased risk for Down's syndrome 1st trimester	Trisomy 21
26	30	12^{+6}	Nuchal translucency 4.3 mm	Normal

2.4. Real-time PCR

The expression of ten AQPs (AQP1, 2, 3, 4, 5, 6, 7, 8, 9, 11) was evaluated by real-time PCR (iQ5, Bio-Rad Laboratories Inc., California, USA) using TaqMan® gene expression assays and TaqMan® 2X PCR Master Mix (Applied Biosystems, Life Technologies Corporation, Carlsbad, California, USA). A list of analysed genes and TaqMan probes is presented in Table 2. Ribosomal 18S gene was used as housekeeping gene.

Real time PCR has been performed with 1 cycle of denaturation of 5 min at 95 °C, followed by 40 cycles of 15 s denaturation at 95 °C and 60 s annealing at 60 °C. The last cycle was followed by 10 min extension at 72 °C.