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Differential Expression of HtrA1 and ADAM12 in Placentas from Preeclamptic and Normotensive Pregnancies

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Abstract

Background: High temperature requirement factor A 1 (HtrA1) and A Disintegrin And Metalloproteinase 12 (ADAM12), which play roles in placental implantation and placental growth, have been implicated in the pathogenesis of preeclampsia.

Methods: We investigated relative mRNA expression of both genes in placental tissues from women with preeclampsia (N = 18) (average gestational age 36 weeks) and an equal number of women with normotensive pregnancies (average gestational age 39 weeks). Real-time polymerase chain reaction was used to measure mRNA extracted from term placental biopsies. Differential gene expression was evaluated using Student's T-test and fold change analyses.

Results: Statistically significant increases in placental HtrA1 (1.69-fold, p = 0.030) and ADAM12 (1.48-fold, p = 0.010) mRNA expression were observed among preeclamptic cases as compared with normotensive controls. HtrA1 expression was correlated with maternal age (p-value< 0.01) among preeclampsia cases.

Conclusion: Increases in HtRA1 and ADAM12 placental gene expression in placentas from preeclamptic pregnancies are consistent with some earlier reports of altered serum protein concentrations in preeclamptic pregnancies. This adds to the literature suggesting that defects in placentation (e.g. involving trophoblast invasion) are of etiologic importance in preeclampsia.

Keywords: Preeclampsia; HtrA1; ADAM12; Placenta; Gene expression

Introduction

The placenta is thought to play a central role in the pathogenesis of preeclampsia [1]. Though clinically characterized by elevated blood pressure and proteinuria in late pregnancy, abnormal placentation, diffuse endothelial dysfunction, and chronic systemic inflammation, likely enhanced directly or indirectly related by a chronically poorly perfused placenta, are important pathophysiological characteristics of preeclampsia [2]. Investigating placental gene expression can potentially enhance understanding of underlying pathophysiological processes in preeclampsia.

HtrA1 high temperature requirement factor A 1 (HtrA1), a member of the serine protease HtrA protein family, has functions related to cell growth, apoptosis and inflammation [3,4]. HtrA1, expressed in both syncytiotrophoblasts and cytotrophoblasts, is thought to play role in placental implantation, placental growth and possibly the pathogenesis of preeclampsia [5,6]. Similarly, a disintegrin and metalloproteinase 12 (ADAM12), a metalloprotease of syncytiotrophoblastic origin involved in placental and fetal growth, has been implicated in the pathogenesis of preeclampsia [7-9]. In sum, HtrA1 and ADAM12 are genes that are of potential importance in trophoblast invasion and normal or abnormal placentation, pathophysiolgic features described in preeclampsia. We investigated differences in mRNA expression of HtrA1 and ADAM12 using a case-control analysis of placental gene expression from 18 preeclamptic mothers and 18 normotensive control mothers. We hypothesized that HtrA1 and ADAM12 expression will be up-regulated in preeclampsia. We also examined whether placental expression of HtrA1 and ADAM12 correlated with selected maternal pre-pregnancy and delivery characteristics.

Materials and Methods

Study population

Study participants, selected among participants of the Omega and Placenta MicroArray studies, described previously [10], were pregnant women who delivered at Swedish Medical Center, Seattle, WA. Preeclampsia was defined as sustained pregnancy-induced hypertension with proteinuria according to American College of Obstetricians and Gynecologists' guidelines [11]. Hypertension was defined as persistent (≥ 2 measures 6 hours apart) blood pressure elevation (>140/90 mm Hg) after 20 weeks gestation. Proteinuria was defined as a sustained (≥ 2 measures 4 hours apart) presence of elevated urine protein (>30 mg/dL or >1+ on a urine dipstick). Controls were selected from among those women who had normotensive pregnancies uncomplicated by proteinuria or gestational diabetes. Women with a history of chronic hypertension, pre-gestational diabetes, and those with multi-fetal pregnancies were in-eligible for inclusion the present study. Placentas were collected from 18 women with preeclampsia and 18 women with normotensive control pregnancies at the time of delivery and medical records were reviewed to abstract information pertaining to maternal and newborn antepartum and perinatal characteristics. All participants provided written informed consent, and all study procedures were approved by the Institutional Review Board of Swedish Medical Center.

Placental sample collection

Placentas were collected weighed, double bagged, transported in

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coolers to our dedicated placenta-processing laboratory, and processed immediately after delivery. In brief, tissue biopsies (~0.5 cm^3 each) were collected from 8 sites (4 maternal and 4 fetal) using a systematic sampling technique to achieve uniformity and adequate sampling [10]. For the present study, tissue biopsies collected from the maternal side of the placenta, consisting primarily of the villous tissue, utero-placental arteries and some decidua basalis, previously implicated in the pathogenesis of preeclampsia, were evaluated [1,2,10]. Biopsies were placed in cryotubes containing RNAlater (Qiagen Inc, Valencia, CA) at 10 µl per 1 mg of tissue and stored at -80°C.

RNA extraction

For the present study, we sampled tissues from each of the four stored biopsies taken from the maternal side of the placenta. Approximately 60 mg of tissue from each biopsy site was cut, weighed and pooled, totaling 240 mg of placental tissues for each subject. The weighted samples were homogenized using a Tissue Tearor (Biospec Products Inc., Bartlesville, OK) or Mini-Beadbeater 8 (Biospec Products Inc. Bartlesville, OK) in a lysis buffer from the RNeasy fibrous Midi Kit (Qiagen Inc, Valencia, CA) with added β-mercaptoethanol to disrupt any proteins that might be destroying nucleic acid. Total RNA was then extracted using a standardized protocol adapted from RNeasy Fibrous Tissue Midi Handbook (Qiagen, Inc., Valencia, CA). Total RNA concentration was calculated by determining absorbance at 260 nm (Spectramax Plus 384 spectrophotometer, Molecular Devices, Sunnyvale, CA). Protein contamination was monitored by the ratio of absorbance at 260 nm to absorbance at 280 nm (A260/A280). All samples had an A260/A280 ratio greater than 1.8. All samples were diluted to 0.25 µg/µl sterile water and were aliquoted for storage at -80°C. All RNA samples, including reference RNAs, underwent a quality control check, and were labeled using the same standardized protocols.

Reverse transcription and real time PCR

We used quantitative real time polymerase chain reaction (QRT-PCR) experiments and Taqman gene expression assays from Applied Biosystems (Applied Bisoystems, Foster City, CA) to measure placental gene expression of HtrA1 (Cat. # Hs01016151) and ADMA12 (Cat. # Hs0022216). First strand cDNA was synthesized using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA).

Characteristics	Preeclampsia Cases (n = 18)	Normotensive Controls (n = 18)	P-values
Age, years*	32.6 (1.8)	30.0 (1.4)	0.26
< 35 years	10 (55.6)	12 (66.7)	0.49
≥ 35 years	8 (44.4)	6 (33.3)	
White	11 (61.1)	12 (66.7)	0.71
GA at delivery, weeks*	35.8 (4.2)	38.9 (1.3)	**<0.01**
< 37 weeks	9 (50.0)	1 (5.6)	**<0.01**
Mode of Delivery			
Vaginal	8 (44.4)	11 (61.1)	0.31
Cesarean	10 (55.6)	7 (38.9)	
Labor	12 (66.7)	13 (72.2)	0.71
Nulliparous	13 (72.2)	10 (55.6)	0.30
Married	14 (77.8)	15 (83.3)	0.18
Pre-pregnancy BMI, kg/m^{2*}	27.0 (1.7)	25.3 (1.5)	0.46
Infant birth weight, kg*	2.6 (0.3)	3.3 (0.1)	**<0.01**
Low birth weight (< 2.5 kg)	8 (44.4)	1 (5.6)	**<0.01**

*Mean (SD), otherwise Number (%)
Abbreviations: BMI: Body Mass Index; GA: Gestational Age; kg: Kilogram

Table 1: Characteristics of Study Population.

QRT-PCR was performed in duplicates using assays developed by Applied Biosystems (Applied Biosystems, Foster City, CA). Reactions were run on an ABI PRISM 7000 Real Time PCR machine (Applied Biosystems, Foster City, CA) using the default cycling conditions. Threshold cycle (Ct) values of the duplicates differing by greater than 0.5 times the standard deviation were re-tested. Ct value duplicates differing by less than 0.5 times the standard deviations were averaged for analysis. Raw measurements were normalized using the geometric mean of SDHA, TBP and YWHAZ genes as previously described by our group [12]. The QRT-PCR experiments were performed at the Center for Perinatal Studies, Swedish Medical Center, Seattle, WA.

Statistical analyses

The distribution of maternal socio-demographic, medical and clinical characteristics according to case-control status was examined. The distribution of continuous variables (e.g., maternal age and infant birth weight) were checked and found to be approximately normal, hence parametric statistical analytical procedures were used when assessing these covariates. Relative expression values for ADAM12 were also normally distributed so parametric statistical tests were performed. However, relative expression values for HtrA1 were highly skewed; hence these values were transformed (using a natural log transformation) before statistical analysis. Comparisons of categorical covariates were made between preeclampsia cases and controls using Chi-squared or Fisher's exacts test. Pair-wise Spearman's correlation coefficient was used to measure the closeness of a linear relationship between relative HtrA1 and ADAM12 gene expression with clinical covariates including maternal age, pre-pregnancy BMI, and infant birth weight. Correlations were examined separately for preeclampsia cases and normotensive controls.

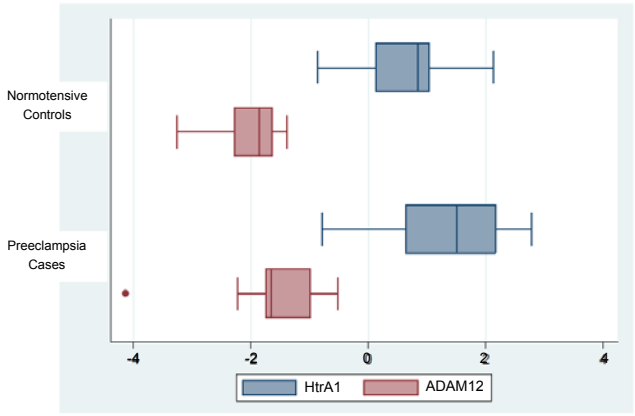
We evaluated differential gene expression values for cases and controls using fold change and Students' T-tests. STATA software was used for the analysis (STATA ver11.0, College Station, TX). All tests were 2-tailed and statistical significance was defines at alpha <0.05.

Results

Characteristics of preeclampsia cases and controls are presented in Table 1. Preeclampsia cases and controls were largely similar with regards to maternal age, race/ethnicity, and marital status. Preeclampsia cases delivered earlier (at 35.8 weeks of gestation compared with 38.9 weeks, p-value <0.01) and were heavier pre-pregnancy (pre-pregnancy BMI of 27.0 kg/m^2 compared with pre-pregnancy BMI of 25.3 kg/m^2) compared with controls. Preeclampsia cases were more likely than controls to deliver infants with low birth weights (44.4% vs. 5.6%, p-value <0.01).

The expression profiles of HtrA1 and ADAM12 are summarized in Figure 1 and Table 2. Mean placental expression of HtrA1 among preeclampsia cases were 1.69-fold higher and statistically different (p-value = 0.030) than mean placental expression of HtrA1 among controls. Similarly, mean placental expression of ADAM12 among preeclampsia cases were 1.48-fold higher and statistically different (p-value-0.010) than mean placental expression of ADAM12 among controls. Correlations between ADAM12 and HtrA1 expression among normotensive controls and preeclampsia cases were 0.31 (p-value=0.20) and 0.42 (p-value=0.08), respectively.

Results of analyses evaluating correlation of HtrA1 and ADAM12 expression with selected maternal pre-pregnancy and delivery characteristics are shown in Table 3. HtrA1 expression was positively and statistically significantly correlated with maternal age among preeclampsia cases (Spearman correlation coefficient 0.77, p-value



Relative placental expression of HtrA1 and ADAM12 (log2transformed)

ot (median and inter-quartile range) depicting placental expression of HtrA1 (blue) and ADAM12 (log transformed) (red) among normotensive controls (upper half) and preeclampsia cases (lower half).

Figure 1: HtrA1 and ADAM12 expression by preeclampsia case control status.

	Preeclampsia cases (N = 18)	Controls (N = 18)	Fold change	P-value*
	Mean ± SE*	Mean ± SE*		
HtrA1**	1.34 ± 0.24	0.64 ± 0.20	**1.63**	**0.030**
ADAM12	0.37 ± 0.04	0.25 ± 0.02	**1.48**	**0.010**

*Student's T-test p-value
**log2-transformed

Table 2: Comparison of placental HtrA1 and ADAM12 expression among preeclamptic cases and controls.

Characteristics	HtrA1		ADAM12	
	Correlation*	P-values	Correlation*	P-values
Normotensive controls				
Maternal age	0.02	0.93	0.32	0.22
Pre-pregnancy BMI	-0.04	0.85	0.31	0.23
Infant birth weight	0.42	0.08	0.21	0.41
Preeclampsia cases				
Maternal age	0.77	**<0.01**	0.16	0.51
Pre-pregnancy BMI	-0.22	0.43	-0.22	0.43
Infant birth weight	-0.34	0.17	-0.04	0.88

*Pair-wise Spearman correlation coefficients
Abbreviations: BMI: body mass index

Table 3: Correlations between placental HtrA1 and ADAM12 expression and selected maternal pre-pregnancy and delivery characteristics.

<0.01). In addition, HtrA1 expression was moderately positively correlated with infant birth weight among normotensive controls, although the correlation did not reach statistically significant (Spearman correlation coefficient 0.42, p-value=0.08). ADAM12 did not appear to be correlated with any of the evaluated characteristics.

Discussion

We observed associations between preeclampsia diagnosis and HtrA1 and ADAM12 mRNA expression in placental tissues collected at delivery. Our findings are consistent with most, but not all, previous reports that suggested associations of HtrA1 and ADAM12 gene expression or their serum protein equivalents with risk of preeclampsia [13,14].

Accumulating evidence suggests that alterations in gene and protein expression of HtrA1, a gene known to regulate placental development, growth and aging through its influence on trophoblast migration, invasion, and extracellular matrix degradation, may be involved in the pathogenesis of preeclampsia [6,13,15,16]. Higher expression of HtrA1 was detected in placental tissues collected from patients with early-onset preeclampsia, compared with those from gestational age matched control samples [13]. HtrA1 protease is a novel inhibitor of TGF-β family members [15], influencing cellular growth and differentiation. HtrA1 is up regulated in both endometrial glands and decidual cells during endometrial preparation for embryo implantation during the first trimester of pregnancy [16]. Investigators have speculated that HtrA1 may regulate trophoblast cell migration and invasion based on findings that show higher levels of HtrA1 expression among less invasive syncytotrophoblasts/cytotrophoblasts and lower levels of HtrA1 expression among invasive syncytotorophoblasts/cytotrophoblasts express lowest levels of HtrA1 [13,16]. Decreased HtrA1 has also been observed in invasive gestational trophoblastic diseases like choriocarcinoma [4] in contrast to what happens during preeclampsia which is characterized by failure of trophoblast invasion. Thus, evidence from our study support potential roles of HtrA1 in trophoblast migration and invasion.

Up regulation of ADAM12 in maternal blood, a growth related transcription factor, has been related to preeclampsia [14]. Similarly, in some studies, higher maternal serum ADAM12 concentration in early pregnancy has been associated with higher risk of developing preeclampsia later in pregnancy [9]. However, early pregnancy plasma ADAM12 concentrations were not associated with risk of preeclampsia in a study by Poon et al. [17]. ADAM12, a component of the TGF-β signaling pathway [18], is known to participate in processing of growth factors (e.g. epidermal growth factor), cytokines, and insulin-like growth factor receptor signaling [18-20]. ADAM12 is also involved in placental leucine aminopeptidase (P-LAP) shedding [8]. P-LAP regulates metalloproteinase 9 activity and trophoblast invasion [21]. These cellular functions have been related to preeclampsia in previous studies.

Investigators have previously suggested to potential roles of HtrA1 and ADAM12 in preeclampsia-related fetal growth restriction [5,22]. For instance, first trimester serum ADAM12 was shown to help predict small for gestational age babies [22]. In secondary analyses, among preeclampsia cases, we compared expression of HtrA1 and ADAM12 in placentas that carried low birth weight and normal birth weight infants. We found higher expression of HtrA1 (log2 HtrA1 1.95 vs. 0.86, p-value=0.02) and ADAM12 (0.43 vs. 0.32, p-value=0.13) among placenta that carried low birth weight infants compared with those that carried normal birth weight infants, although only the HtrA1 comparison was statistically significant. Due to sample size issues, we could not perform similar assessment among controls. Functions of both HtrA1, which has an IGF-binding domain [23], and ADAM12 that influences activities of growth factors may account for these potential associations.

Some limitations of our study deserve mention. First, this cross-sectional study is unable to clearly elucidate whether increased HtrA1 and ADAM12 mRNA expression functions in preeclampsia pathogenesis or are themselves induced by disease-related metabolic changes. Studies documenting altered maternal plasma concentrations of protein products from these genes, however, suggest that alternation in protein expression (and possibly expression at the genomic level) may precede the clinical diagnosis of preeclampsia [14]. New studies that examine mRNA expression profiles in placental tissues remaining after chorionic villus sampling (CVS) may directly address questions

about the temporality of altered gene expression of candidate genes in preeclamptic and normotensive pregnancies. Second, although we biopsied placental tissues from multiple sites, likely heterogeneity in cell types and cell populations may have influenced our results. Notably, Lorenzi and colleagues have reported that HtrA1 mRNA expression varies across the various regions of the placenta [5].

In this study, we found up-regulation of placental HtrA1 and ADAM12 gene expression among preeclampsia cases compared with controls. Our findings complement and extend previous clinical and basic science research based earlier reports of HtrA1 or ADAM12 related altered serum protein concentrations in preeclampsia; expression deregulation in pathways that have been related to preeclampsia; and, other smaller expression studies, by demonstrating alterations in placental expression of HtrA1 and ADAM12 among an epidemiologically well-characterized study population. Our findings highlight the potential importance of these genes in defects of placentation. Future studies investigating post-transcription regulation, downstream protein expression measurements, and expression in tissue obtained in early pregnancy will further enhance our understanding of the role of these genes and related biomarkers in preeclampsia.

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