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Expression and Localization of COMMD1 Proteins in Human Placentas from Women with Preeclampsia

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Purpose: Recently, COMMD1 has been identified as a novel interactor and regulator of hypoxia-inducible factor-1 and nuclear factor kappa B transcriptional activity. The goal of this study was to determine the difference of COMMD1 expression in the placentas of women with normal and preeclamptic (PE) pregnancies. **Materials and Methods:** Immnoperoxidase and immunofluorescent staining for COMMD1 was performed on nine normal and nine severe PE placental tissues, and COMMD1 mRNA expression was quantified by quantitative reverse transcription polymerase chain reaction. **Results:** The expression of mRNA of COMMD1 was significantly higher in the study group than in the control group. The immunoreactivity was higher especially in the syncytiotrophoblast of PE placentas than in the control group. **Conclusion:** This study demonstrated increased placental COMMD1 expression in women with severe preeclampsia compared to that found in women with normal pregnancies, and this finding might contribute to a better understanding of the pathophysiology of preeclampsia.

Key Words: COMMD1, placenta, preeclampsia

INTRODUCTION

Preeclampsia (PE) occurs in 5% of human pregnancy, and could cause serious maternal and perinatal morbidity and mortality, without clear understanding of its pathogenesis. Recent studies have shown that excessive maternal systemic inflammatory response to pregnancy and uteroplacental hypoxia might play a major role in inducing endothelial dysfunction,[1,2] which is considered to be responsible for the pathogenesis of PE by leading to cellular activation and damage.[3-6] COMMD1 is the prototype of copper metabolism gene MURR1 domain (COMMD) protein family.[7] Till now, 10 family members have been discovered sharing 70 to 85 amino acids unique to COMMD, without definite functions in human. Several recent reports have implied that COMMD1 may play a role in various cellular processes and interact with some components of the nuclear factor kappa B (NF-κB) signaling pathway.[7-9] This domain might be involved in protein-protein interactions implicating a novel protein-protein interaction motif.[7] COMMD1 inhibits the NF-κB transcriptional activity that promotes the expression of gene products involved in

several cellular processes, including cell survival, inflammation, viral replication, and oncogenesis.[10-13] Furthermore, COMMD1 has been identified as a novel interactor and regulator of hypoxia-inducible factor-1 (HIF-1) activity.[14] HIF-1α is the major transducer of hypoxia signaling in several tissues, including human placenta.[15,16] Increased HIF-1 activity has also been associated with preeclampsia.[16] Recently, it has been shown that COMMD1 is normally expressed in human placenta, localized within the placental villi, and present in the syncytiotrophoblast (SCT), cytotrophoblast, vascular endothelial cells, and Hofbauer cells.[17] Given the role of COMMD1 in inflammation and hypoxic damage, it is possible to hypothesize that the expression pattern of COMMD1 may be changed in the preeclamptic condition. Therefore, this study was designed to determine the difference of COMMD1 expression in the placentas of women with normal and preeclamptic pregnancies.

MATERIALS AND METHODS

Sample collection

Placentas from 9 patients with severe PE and 9 control women were collected at the time of their cesarean section at Konkuk University Hospitals. To standardize collection, the same investigator collected all samples, and central portions of placentas were collected after placental delivery. The control subjects were normotensive pregnant women who were admitted for elective cesarean section or delivery. Collection and processing of human placentas were approved by the institutional review board, and informed consent was obtained from each patient. PE was defined as hypertension (systolic blood pressure ≥140 mm Hg and diastolic blood pressure ≥90 mm Hg after 20 weeks' gestation) and proteinuria (≥300 mg in a 24 hr urine collection or one dipstick measurement of ≥1+) according to the criteria of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy.[18] Severe PE was diagnosed on the basis of systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥110 mm Hg, significant proteinuria (≥5 gm per 24-hour urine collection or dipstick measurement of ≥3+), or the presence of severe symptoms such as headache, visual disturbances, upper abdominal pain, oliguria, convulsion, elevated serum creatinine, thrombocytopenia, marked liver enzyme elevation, and pulmonary edema. Multiple pregnancies, presence of maternal chronic hypertension, cardiovascular disease, renal disease, hepatic disease, diabetes, or other infectious or autoimmune diseases were excluded from the study.

Extraction of total RNA and reverse transcription

Total RNA was extracted from placental tissue. The extraction was performed according to the manufacturer's protocol, and 1 mg of total RNA was used in reverse transcription under conditions recommended by the manufacturer.

Quantitative reverse transcription polymerase chain reaction (RT-PCR)

COMMD1 mRNAs and the internal standard [glyceraldehydes 3-phosphate dehydrogenase (GAPDH)] expressions were quantified by real-time polymerase chain reaction (PCR). The PCR was performed using the primers 5'-CT GGAGGCATTCTTGACTGCTC-3'and 3'-GCTCTCAC GGATTTTTGTCTTGTG-5'. PCR conditions were as described by Hoffmann, et al.[19] The results were normalized to GAPDH expression levels.

Immunohistochemical staining

COMMD1 protein was detected with immnoperoxidase and immunofluorescent staining. Paraffin embedded tissues were sectioned in 5 μm thickness. The sections were deparaffinized and rehydrated using xylene and alcohol. The sections were pretreated for 10 min in a microwave oven for antigen retrieval and then incubated at room temperature for 30 min. After rinses, sections were incubated in 0.5% H_2O_2 in PBS (pH7.4) for 20 minutes to inhibit endogenous peroxidase activity. Sections were then reacted with 10% normal goat serum for 1 hour to block nonspecific binding. They were then incubated in 1 : 100 dilution of primary antibody (Purified Mouse Anti Human COMMD1 Monoclonal antibody: Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4°C. After rinses, sections were incubated with the biotinylated secondary antibodies at 1 : 250 for 1 hour at room temperature. Sections were then treated with an avidin-biotin-peroxidase complex (Vectastain ABC mous Elite kit; Vector Laboratories, Burlingame, CA, USA) by following the manufacturer's manual. The reaction was visualized using a solution containing 0.0125% diaminobenzidine and 0.005% hydrogen peroxide. After rinses, sections were counterstained with hematoxylin and mounted with mounting medium.

In immunofluorescent staining, sections were incubated with primary antibody, similar to that of the immunoperoxidase staining. After rinses, sections were incubated in 1 : 200

Table 1. Clinical Characteristics of Preeclampsia and Normal Control Subjects

	Preeclampsia (n=9)	Normal (n=9)	*p* value
Age (yrs)	30.1±4.3	32.6±4.5	0.25
GA at delivery (wks)	33.9±3.1	37.9±2.0	0.005
Systolic BP (mm Hg)	170.6±11.8	106.1±11.1	<0.00001
Diastolic BP (mm Hg)	117.8±7.1	58.3±5.0	<0.00001
Proteinuria	9/9	None	
Neonatal birth weight (grams)	1935±825	3165±501	0.0015

GA, gestational age; BP, blood pressure; SD, standard deviation.
Values are mean±SD.

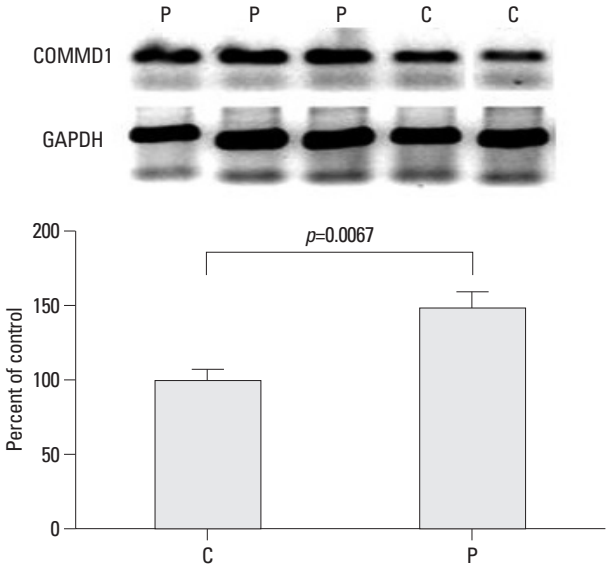

Fig. 1. COMMD1 mRNA expression in normal human placentas and preeclamptic placentas, COMMD1 mRNA of demonstrated significant increase in preeclamptic placenta (p=0.0067) in reverse transcriptase-polymerase chain reaction. Data are means±SD of densitometry measurements relative to the results obtained in placentas of control group (control set at 100%). GAPDH, glyceraldehyde 3-phosphate dehydrogenase; C, control; P, preeclamptic pregnancy; SD, standard deviation.

dilution of secondary antibody (Donky anti-mouse Alexa 594, Invitrogen, Carlsbad, CA, USA) for 1 hour at room temperature. The sections were rinsed with PBS and then mounted by using mounting medium (S3023 non-fluorescent mounting medium; Dako, Carpinteria, CA, USA).

Photographs were obtained with 2048×1536 pixel digital CCD camera (DP70, Olympus, Tokyo, Japan) for immunohistochemical images, and fluorescent microscope (BX61-32FDIc, Olympus, Tokyo, Japan) for immunofluorescent images. Images were analyzed and each cell was semi-quantitatively scored for the expression of COMMD1 as follows: 0: no staining, 1: weak intensity, 2: moderate intensity, 3: high intensity.

Densitometry

The membranes were visualized with densitometric scanning using the densitometer (IMAGE READER LAS-1000 lite, Fuji Photo Film Co. Ltd., Tokyo, Japan). Densitometry was carried out with digital analysis software (Fuji Photo Film Co. Ltd., Tokyo, Japan).

Statistical analyses

Statistical analyses were performed with dBSTAT, version 4.0 (dBSTAT Inc., Seoul, Korea). Data were expressed as mean±standard deviation. Patients' characteristics and statistical differences for densitometric data in RT-PCR between the two groups were compared by using a Student t-test. Multiple linear regression analysis was used to examine the relationship between COMMD1 and relevant variables. A p-value of <0.05 was considered significant.

RESULTS

Demographic and clinical data from the studied subjects are given in Table 1. There was no significant difference in maternal age between the normal and PE groups. In the PE group, systolic and diastolic blood pressures were significantly higher, and gestational age at delivery and birth weight were lower. The expression of COMMD1 mRNA was significantly higher in the study group than in the control group (p=0.0067) (Fig. 1). Multiple linear regression analysis was used to find whether the levels of COMMD1 mRNA were significantly different, regardless of gestational age at delivery and neonatal birth weight. Only PE was independently related to placental COMMD1 mRNA levels (p=0.03). COMMD1 protein was stained in endothelial cells of chorionic villi vessels, cytotrophoblasts, SCTs, stroma, and deciduas in both groups, but the immunoreactivity was especially higher in the SCT of preeclamptic placentas than in the control group (Fig. 2). The staining scores were significantly higher in the SCT and cytotrophoblast of the PE group than in the control group (Table 2). COMMD1 was

over-expressed in both the cytoplasm and membrane of the SCT, which is shown in red color in Fig. 3. No significant difference was found in the COMMD1 signals in other tissues between the groups.

DISCUSSION

The exact functions of COMMD have not yet been defined

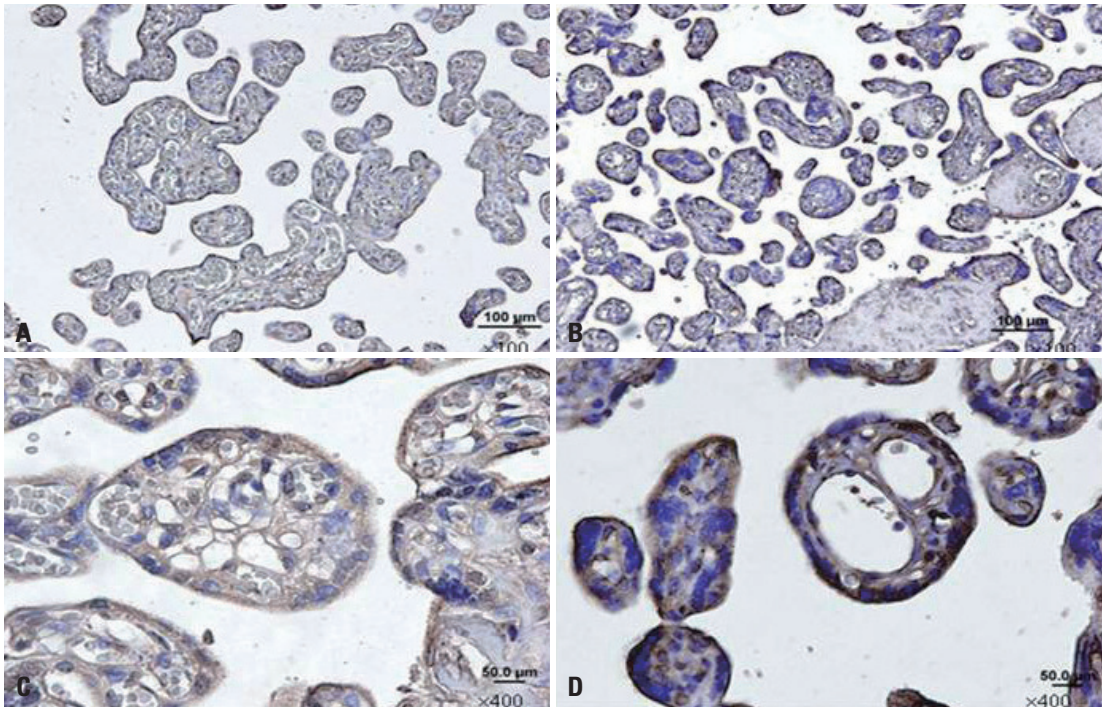

Fig. 2. Immnoperoxidase staining for COMMD1 in the placentas. The intensity of staining for COMMD1 was increased significantly in syncytiotrophoblast of preeclamptic placenta. (A and C) Normal placenta. (B and D) Preeclamptic placentas.

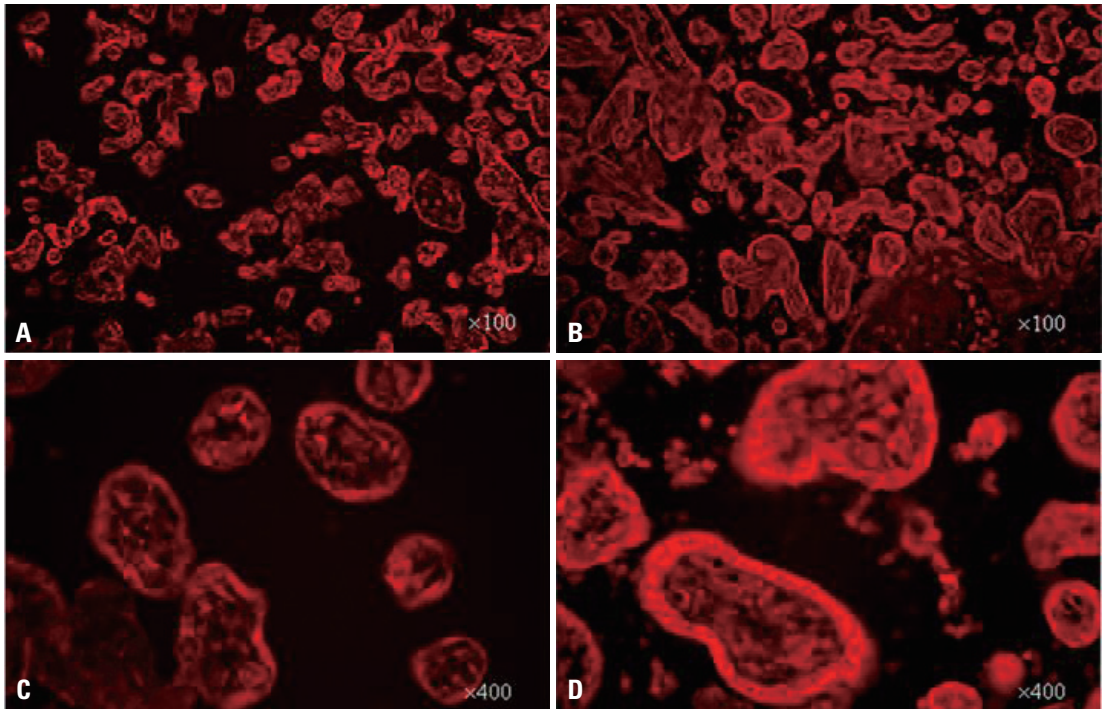

Fig. 3. Immunofluorescent staining of COMMD1 in normal and preeclamptic placenta. The red signal of COMMD1 was significantly increased in preeclamptic placenta. The increased signal in preeclamptic placenta was mostly distributed in the cytoplasm and membrane of syncytiotrophoblast. (A and C) Normal placenta. (B and D) Preeclamptic placentas.

Table 2. Tissue Distribution and Staining Intensity of Cellular COMMD1 in Normal and Preeclamptic Placentas

Immunoreactive tissues	Preeclampsia (n=9)	Normal (n=9)
Chorionic villi vessle		
Endothelial cell	1-2	1-2
Smooth muscle	0	0
Cytotophoblast	2-3	0-1
Syncytiotrophoblast	3	0-1
Stroma	0-1	0-1

0, no staining; 1, weak intensity; 2, moderate intensity; 3, high intensity.

till now. Nevertheless, it is known to be involved in copper transport and homeostasis by binding ATP7B in copper metabolism.[20] Copper activates several proangiogenic factors such as vascular endothelial growth factor,[21-23] which leads to proliferation and migration of endothelial cells, thus contributes to formation of blood vessels. Therefore, copper is considered to play an important role in successful development of the placenta and the fetus. Furthermore, an animal study showed that COMMD1$^{-/-}$ mice were embryonically lethal, their development was generally retarded, and placenta vascularization was absent.[14] Recently, COMMD1 has been reported to be found in the SCTs, cytotrophoblasts, and vascular endothelial cells of human placenta throughout all periods of gestation; it has been especially found in higher levels during 10-12 weeks of gestation.[17] This indicates that COMMD1 participates in human copper homeostasis and establishes fetomaternal circulation, after which its expression is decreased.

As mentioned above, COMMD1 is a regulator of HIF-1 activity. Several studies suggest that HIF-1α, along with two of the numerous genes that it regulates, soluble fms-like tyrosine kinase 1[24] and soluble endoglin,[25] are over-expressed in preeclamptic women and play a key role in the development of PE.[26-29] Thus, overexpression of the COMMD1 protein in the later gestation period of PE patients compared to the control group in this study, might be a compensatory mechanism against the preeclamptic change.

Another function of COMMD1 is to inhibit the NF-κB signal pathway. COMMD proteins are known to negatively regulate the expression of a number of pro-inflammatory NF-κB-inducible genes.[9] In response to tumor necrosis factor (TNF) stimulation, COMMD1-deficient cells accumulate higher amounts of mRNA of κB-dependent genes such as intercellular adhesion molecule 1 (ICAM-1) and chemokine ligand 2.[9] Furthermore, a previous study suggested that serum from patients with PE stimulated the expression of ICAM-1 on trophoblasts, of which the expression could in turn stimulate maternal immunological recognition and rejection reactions and result in disrupted trophoblast trafficking, thereby causing incomplete placentation and leading to PE.[30] These findings also support the idea that COMMD1 is over-expressed in preeclamptic placentas in a compensatory mechanism against the inflammatory reactions led by various cytokines and adhesion molecules. Several proteins that are thought to be increased in PE patients by a compensatory mechanism are nuclear factor erythroid 2-related factor 2, and clusterin.[31,32] COMMD1 could also be a good candidate for a predictor of preeclampsia, since it is overexpressed throughout gestation. Also, if the overexpression is clearly defined as a compensatory mechanism, this could be exploited in the prevention and treatment of PE. The limitations in this study are lack of maternal serum data and small sample size. Also, the origin of overly expressed COMMD1 in the SCT is not clear. Since SCTs are in direct contact with maternal blood, syncytial staining detected by immunohistochemistry could be due to maternal COMMD1 bound to the surface of chorionic villi. Further studies using maternal serum and selective RT-PCR are needed. Furthermore, there was significant difference in the gestational age at the time of placenta sampling, which was a confounder to the difference in the expression of COMMD1. Therefore, multiple linear regression analysis was used to see whether the levels of COMMD1 mRNA were significantly different, regardless of gestational age at delivery. Nonetheless, to the best of our knowledge, this is the first study to show increased expression of COMMD1 in the placentas of severe PE patients. In conclusion, this study demonstrated increased placental COMMD1 expression in women with severe preeclampsia compared to that found in women with normal pregnancies. Finally, this finding might contribute to a better understanding of the pathophysiology of PE, which is mainly explained by the imbalance of angiogenic/anti-angiogenic factors in hypoxic conditions.

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