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

Han-Sung Kwon,1 Seung-Hwa Park,2 Han-Sung Hwang,1 In-Sook Sohn,1 and Soo-Nyung Kim1

Departments of 1Obstetrics and Gynecology, 2Anatomy, Konkuk University School of Medicine, Seoul, Korea.

Received: January 10, 2012
Revised: February 21, 2012
Accepted: February 21, 2012
Corresponding author: Dr. Han-Sung Hwang, Department of Obstetrics and Gynecology, Konkuk University School of Medicine, 120-1 Neungdong-ro, Gwangjin-gu, Seoul 143-729, Korea. Tel: 82-2-2030-7645, Fax: 82-2-2030-7747 E-mail: 20050024@kuh.ac.kr

- The authors have no financial conflicts of interest.

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: Immunoperoxidase 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. Furthermore, COMMD1 has been identified as a novel interactor and regulator of hypoxia-inducible factor-1 (HIF-1) activity. HIF-1α is the major transducer of hypoxia signaling in several tissues, including human placenta. Increased HIF-1 activity has also been associated with preeclampsia. 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. 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 placenats 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 the 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. Severe PE was diagnosed on the basis of systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥110 mm Hg, significant proteinuria (≥5 g/day in 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’-GGAGGTCATTTGTGTG-3’and 3’-GCTCTTTCACGATTTCCTTGTCTTG-5’. PCR conditions were as described by Hoffmann, et al. The results were normalized to GAPDH expression levels.

Immunohistochemical staining
COMMD1 protein was detected with immunoperoxidase 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% H2O2 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 to block nonspecific binding. Sections were then reacted with 10% normal goat serum for 1 hour to block nonspecific binding. Sections were then treated with an avidin-biotin-peroxidase complex (Vestastain 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
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

---

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.

**Fig. 2.** COMMD1 protein expression in normal and preeclamptic placentas, showing the immunoreactivity was especially higher in the SCT of preeclamptic placentas than in the control group. The staining scores were significantly higher in the SCT and cytotrophoblast of the PE group than in the control group (Table 2). COMMD1 was

---

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).
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...
null
ence of endothelial cell dysfunction in the pregnancy syndrome preeclampsia. Am J Hypertens 1991;4:700-8.
2. Dekker GA, Sibai BM. Etiology and pathogenesis of preeclampsia: current concepts. Am J Obstet Gynecol 1998;179:1359-75.
3. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol 1999;180(2 Pt 1):499-506.
4. Sacks G, Sargent I, Redman C. An innate view of human pregnancy. Immunol Today 1999;20:114-8.
5. Khong TY, Robertson WB. Spiral Artery Disease. In: Coulam CB, Faulk WP, McIntyre JA, editors. Immunological obstetrics. New York: W.W.Norton & Company; 1992. p.492-501.
6. Conrad KP, Benyo DF. Placental cytokines and the pathogenesis of preeclampsia. Am J Reprod Immunol 1997;37:240-9.
7. Burstein E, Hoberg JE, Wilkinson AS, Rumble JM, Csomos RA, Komarck CM, et al. COMMD proteins, a novel family of structural and functional homologs of MURR1. J Biol Chem 2005;280:22222-32.
8. Ganesh L, Burstein E, Guha-Niyogi A, Louder MK, Mascola JR, Klomp LW, et al. The gene product Murr1 restricts HIV-1 replication in resting CD4+ lymphocytes. Nature 2003;426:853-7.
9. Maine GN, Mao X, Komarck CM, Burstein E. COMMD1 promotes the ubiquitination of NF-kappaB subunits through a cullin-containing ubiquitin ligase. EMBO J 2007;26:436-47.
10. Green DR. Death and NF-kappaB in T cell activation: life at the edge. Mol Cell 2003;11:551-2.
11. Karin M, Lin A. NF-kappaB at the crossroads of life and death. Nat Immunol 2002;3:221-7.
12. Perkins ND. The Rel/NF-kappaB family: friend and foe. Trends Biochem Sci 2000;25:434-40.
13. Silverman N, Maniatis T. NF-kappaB signaling pathways in mammalian and insect innate immunity. Genes Dev 2001;15:2321-42.
14. van de Sluis B, Muller P, Duran K, Chen A, Groot AJ, Klomp LW, et al. Increased activity of hypoxia-inducible factor 1 is associated with early embryonic lethality in Commd1 null mice. Mol Cell Biol 2007;27:4142-56.
15. Semenza GL, Wang GL. A nuclear factor induced by hypoxia via de novo protein synthesis binds to the human erythropoietin gene enhancer at a site required for transcriptional activation. Mol Cell Biol 1992;12:5447-54.
16. Rajakumar A, Doty K, Daftary A, Harger G, Conrad K. Impaired oxygen-dependent reduction of HIF-1alpha and -2alpha proteins in pre-eclamptic placentas. Placenta 2003;24:199-208.
17. Donadio S, Alfaiady N, De Keuckeleire B, Micoud J, Feige JJ, Challis JR, et al. Expression and localization of cellular prion and COMMD1 proteins in human placenta throughout pregnancy. Placenta 2007;28:907-11.
18. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000;183:S1-22.
19. Hoffmann P, Feige JJ, Alfaiady N. Expression and oxygen regulation of endothrine gland-derived vascular endothelial growth factor/prokineticin-1 and its receptors in human placenta during early pregnancy. Endocrinology 2006;147:1675-84.
20. Tao TY, Liu F, Klomp L, Wijmenga C, Gitlin JD. The copper toxicity gene product Murr1 directly interacts with the Wilson disease protein. J Biol Chem 2003;278:41593-6.
21. Lowndes SA, Harris AL. Copper chelation as an antiangiogenic therapy. Oncol Res 2004;14:529-39.
22. Nasulewicz A, Mazur A, Opolski A. Role of copper in tumour angiogenesis--clinical implications. J Trace Elem Med Biol 2004;18:1-8.
23. Pan Q, Kleer CG, van Golen KL, Irani J, Bottema KM, Bias C, et al. Copper deficiency induced by tetrathiomolybdate suppresses tumor growth and angiogenesis. Cancer Res 2002;62:4854-9.
24. Ahmed A, Dunk C, Ahmad S, Khalil A. Regulation of placental vascular endothelial growth factor (VEGF) and placenta growth factor (PIGF) and soluble Flt-1 by oxygen--a review. Placenta 1997;21 Suppl A:S16-24.
25. Sánchez-Elsner T, Botella LM, Velasco B, Langa C, Bernabéu C. Endoglin expression is regulated by transcriptional cooperation between the hypoxia and transforming growth factor-beta pathways. J Biol Chem 2002;277:43799-808.
26. Rajakumar A, Whitelock KA, Weissfeld LA, Daftary AR, Markovic N, Conrad KP. Selective overexpression of the hypoxia-inducible transcription factor, HIF-2alpha, in placentas from women with preeclampsia. Biol Reprod 2001;64:499-506.
27. Caniggia I, Winter JL, Adriana and Luisa Castellucci Award lecture 2001. Hypoxia inducible factor-1: oxygen regulation of trophoblast differentiation in normal and pre-eclamptic pregnancies-a review. Placenta 2002;23 Suppl A:S47-57.
28. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of preeclampsia. N Engl J Med 2004;350:672-83.
29. Foidart JM, Schaaps JP, Chantraire F, Munaut C, Lorquet S. Dysregulation of anti-angiogenic agents (sFlt-1, PLGF, and sEndoglin) in preeclampsia—a step forward but not the definitive answer. J Reprod Immunol 2009;82:106-11.
30. Abe E, Matsubara K, Oka K, Kusanagi Y, Ito M. Cytokine regulation of intercellular adhesion molecule-1 expression on trophoblasts in preeclampsia. Gynecol Obstet Invest 2008;66:27-33.
31. Wruck CJ, Huppertz B, Bose P, Brandenburg LO, Pufe T, Kadyrov M. Role of a fetal defence mechanism against oxidative stress in the aetiology of preeclampsia. Histopathology 2009;55:102-6.
32. Shin JK, Han KA, Kang MY, Kim YS, Park JK, Choi WJ, et al. Expression of clusterin in normal and preeclamptic placentas. J Obstet Gynaecol Res 2008;34:473-9.