Available online at www.sciencedirect.com
ScienceDirect
PLACENTA
Placenta 28 (2007) 487–497

# Microarray Analysis of Differentially Expressed Fetal Genes in Placental Tissue Derived from Early and Late Onset Severe Pre-eclampsia

H. Nishizawa [a,b,*,1], K. Pryor-Koishi [a,b,1], T. Kato [b,c], H. Kowa [b,c], H. Kurahashi [b,c], Y. Udagawa [a]

[a] *Department of Obstetrics and Gynecology, Fujita Health University, Toyoake, Japan*
[b] *21st Century COE Program, Development Center for Targeted and Minimally Invasive Diagnosis and Treatment, Fujita Health University, Toyoake, Japan*
[c] *Division of Molecular Genetics, Institute for Comprehensive Medical Science, Fujita Health University, Toyoake, Japan*

Accepted 12 May 2006

**Abstract**

Although it has been well documented that pre-eclampsia is caused by a combination of maternal and fetal susceptibility genes, little is known about the precise etiology of this complicated disorder. To investigate how the expression of fetal genes contributes to the mechanisms underlying the progression of this disease, we have analyzed differentially expressed genes using placentas from 13 normal pregnancies and 14 pregnancies with severe pre-eclampsia. We performed genome-wide expression profiling using high-density oligonucleotide microarrays, followed by validation using real-time PCR. Among the 47,000 genes that were screened in the microarray, 137 genes were found to be differentially expressed between normal and pre-eclamptic tissues. Among these candidates, 70 were up-regulated and 67 were down-regulated. The up-regulated genes included leptin and inhibin A, which are well-known biological markers for pre-eclampsia, as well as FLT1, which was recently proved to be tightly linked with the etiology of this disease. Gene ontology analysis further revealed several biological processes that could be associated with the development of pre-eclampsia, including response to stress, host–pathogen interactions, lipid metabolism, and carbohydrate metabolism. Analyses of biological mechanisms highlighted some important pathways that may be involved in this disorder, such as the TGF-β and CEBPA-related pathways. Furthermore, when our present subjects were classified as either severe cases of early onset or late onset pre-eclampsia, the expression of 11 genes could be correlated with the severity of this disorder. These genes may therefore prove to be novel biological markers by which the severity of this condition could be predicted. Our data are likely to be a useful future resource in the elucidation of the disease-process and in the identification of novel markers for pre-eclampsia.


*Keywords:* Microarray analysis; Gene ontology; Pre-eclampsia

## 1. Introduction

Pre-eclampsia is one of the most serious pregnancy-associated disorders, and is defined by hypertension with proteinuria [1]. It is not a simple complication of pregnancy, but is rather a syndrome of multiple organ failure involving the liver, kidney, and lung, as well as coagulatory and neural systems. There is now an emerging consensus that pre-eclampsia is a complex polygenetic trait in which maternal and fetal genes, as well as environmental factors, are involved, although the precise mechanism of the disorder has remained elusive [2]. Accumulating evidence has indicated, however, that placental dysfunction, endothelial dysfunction, oxidative stress and the inflammatory response may participate in the development

* Corresponding author at: Fujita Health University, 1-98 Dengakugakubo, Kutsukake, Toyoake, Aichi, 470-1192, Japan. Tel.: +81 562 93 9294; fax: +81 562 95 1821.
*E-mail address:* nharuki@fujita-hu.ac.jp (H. Nishizawa).
[1] These authors contributed equally to this work.

0143-4004/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.placenta.2006.05.010

of this disorder. Indeed, several disease-susceptible genes were recently identified by analysis of polymorphic markers [3,4].

Expression profiling using microarray has proved to be a powerful tool for studying complex disorders such as pre-eclampsia. Patients with pre-eclampsia recover from clinical symptoms of the disorder just after the delivery of the fetus and placenta. Pre-eclampsia also occurs in patients with a hydatidiform mole in the absence of any tissues of fetal origin. Taken together, there is an emerging consensus that the placenta plays an essential role in the etiology of this disorder. To date, there have been several reports that have addressed the gene expression profiles of the placentas from pre-eclamptic patients. These studies have shown that genes such as the obesity-related genes, cytokine-receptor genes, and apoptosis-related genes are essential for the development of pre-eclampsia [5–7].

Since the prognosis of both the mother and fetus in cases of severe pre-eclampsia is poorer than generally expected, this disorder is regarded as potentially very dangerous by gynecologists. However, the scarcity of early biomarkers for pre-eclampsia has hindered our ability to launch preventive and therapeutic measures to treat this disease in a timely manner. In addition, although no diagnostic criteria for the severity of pre-eclampsia are currently defined, there is little doubt that an early-onset is detrimental to the overall prognosis. Since the risks of maternal multi-organ dysfunction and fetal distress are higher during an early onset, it has been recommended that the fetus should be delivered at gestational week 32–34 in these cases [8–10]. It is significant that in early onset, and before gestational week 32, maternal mortality is 20 times higher than in cases of normal term delivery [11]. Moreover, analyses of leukocyte function and serum cytokine levels now indicate that early onset pre-eclampsia is a different subset of the disorder from late onset [12]. Furthermore, women with a history of early onset pre-eclampsia show an increased risk of chronic hypertension or ischemic heart disease leading to earlier future cardiovascular death [13–15]. Thus, it is of vital clinical importance to compare the expression profiles between the early onset and late onset forms of this disease and thereby increase our understanding of their respective etiologies and aid in the future development of new therapies.

In this study, we have performed such gene expression profiling to further elucidate the mechanisms underlying the development of the pre-eclamptic condition. We washed the samples extensively to avoid contamination of maternal blood. We thus compared the expression profiles in placentas from women who underwent a normal pregnancy and from women suffering from severe pre-eclampsia. In addition, we compared the expression data between the early and late onset forms of pre-eclampsia and obtained a number of potential new prognostic biomarkers for this disease.

## 2. Materials and methods

### 2.1. Human subjects

All of the clinical samples were collected at the Department of Obstetrics and Gynecology, Fujita Health University, Japan. Placental biopsies were obtained from both normotensive patients and from those with pre-eclampsia ($n = 14$) (early onset type; earlier than 31 weeks gestation, $n = 6$ and late onset type; 31 weeks gestation or later, $n = 8$). Pre-eclampsia was defined as a blood pressure of higher than 160/110 mmHg, with proteinuria of more than 2 g in a 24 h collection. Normotensive subjects ($n = 13$) were matched for maternal and gestational ages, and for body mass index during pre-pregnancy (Table 1). However, pre-eclamptic women delivered newborns with lower birth weight percentiles (Mann–Whitney $U$-test; $P = 0.002$). Informed consent was obtained from each patient. The study was approved by the Ethical Review Board for Human Genome Studies at Fujita Health University.

### 2.2. Placental biopsy collection

All of the placental biopsies both from pre-eclampsia and normal pregnancy were obtained after Caesarean sections. To avoid the further effect of labor on the expression profile, only placental samples that were obtained from the women who had not undergone labor were included in the study. A central area of chorionic tissue was dissected, and the maternal deciduas and amnionic membranes were removed. We then dissected 1-cm-thick sections of placental vili from the central area between basal and chorionic plates. After vigorous washing of the maternal blood with saline, tissues were immediately frozen in liquid nitrogen and stored until use.

### 2.3. RNA extraction

Total RNA was extracted from the chorionic villous tissues with an RNeasy mini-kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. The quality of the RNA samples was determined by electrophoresis through denaturing agarose gels and staining with ethidium bromide. The RNA was quantified and evaluated for purity by UV spectrophotometry. To assess the quality of the RNA further, all specimens were tested by expression analysis of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), using conventional RT-PCR. To test for possible contamination by maternal blood, the expression level of leukocyte-specific gene (leukocyte common antigen, LCA) was also examined using conventional semi-quantitative RT-PCR. We performed PCR within the linear amplification range. The products were electrophoresed in 2% agarose gel containing ethidium bromide, which were then visualized with ultraviolet.

### 2.4. Microarray analysis

The expression profiles of approximately 47,669 genes were analyzed using a Whole Human Genome Oligo Microarray Kit (Agilent Technologies). A total of 10 placentas from women with pre-eclampsia and four from normal subjects were used as test samples in the hybridizations. Reverse transcription labeling and hybridization were performed using the protocol recommended by the manufacturer. A test RNA sample purified from each normal or pre-eclampsia placenta was individually used for microarray analysis (Cy5-labeled), with pooled RNA derived from normal placentas as a template control (Cy3-labeled)

Table 1
Clinical parameters in the study groups

| | Normal pregnancy ($n = 24$) | Severe pre-eclampsia ($n = 21$) | $P$ value |
|---|---|---|---|
| Gestational age (weeks) | 32.9 ± 5.6 | 32.9 ± 4.0 | n.s. |
| Maternal age (years) | 29.2 ± 5.9 | 30.3 ± 3.7 | n.s. |
| Systolic BP (mmHg) | 113.1 ± 10.9 | 170.6 ± 14.6 | <0.05 |
| Diastolic BP (mmHg) | 67.1 ± 10.2 | 105.7 ± 14.5 | <0.05 |
| Poteinuria (%)[a] | 0 | 100 | <0.05 |
| Body mass index[b] | 22.9 ± 5.2 | 22.1 ± 3.1 | n.s. |
| Birth weight (g) | 2265.9 ± 966.6 | 1533.7 ± 685.7 | <0.05 |
| Placental weight (g) | 616.4 ± 193.3 | 277.0 ± 66.1 | <0.05 |

[a] ⩾2 g in a 24 h collection.
[b] Pre-pregnancy.