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## Increased decidual mRNA expression levels of candidate maternal pre-eclampsia susceptibility genes are associated with clinical severity

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### Abstract

**Introduction**—Pre-eclampsia (PE) has a familial association, with daughters of women who had PE during pregnancy having more than twice the risk of developing PE themselves. Through genome-wide linkage and genetic association studies in PE-affected families and large population samples, we previously identified the following as positional candidate maternal susceptibility

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#### Author Contributions

HEJ Yong: I declare that I participated in the substantial contribution to acquisition, analysis and interpretation of data, drafting and revision of the manuscript and that I have seen and approved the final version. I have no conflicts of interest.

P Murthi: I declare that I participated in the substantial contribution to conception and design, data analysis and interpretation, drafting the manuscript and critical revision for intellectual content and that I have seen and approved the final version. I have no conflicts of interest.

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genes for PE; *ACVR1*, *INHA*, *INHBB*, *ERAP1*, *ERAP2*, *LNPEP*, *COL4A1* and *COL4A2*. The aims of this study were to determine mRNA expression levels of previously identified candidate maternal pre-eclampsia susceptibility genes from normotensive and severe PE (SPE) pregnancies and correlate mRNA expression levels with the clinical severity of SPE.

**Methods**—Third trimester decidual tissues were collected from both normotensive (n=21) and SPE pregnancies (n=24) and mRNA expression levels were determined by real-time PCR. Gene expression was then correlated with several parameters of clinical severity in SPE. Statistical significance was determined by Mann-Whitney *U* test and Spearman's Correlation.

**Results**—The data demonstrate significantly increased decidual mRNA expression levels of *ACVR1*, *INHBB*, *ERAP1*, *ERAP2*, *LNPEP*, *COL4A1* and *COL4A2* in SPE ( $p < 0.05$ ). Increased mRNA expression levels of several genes – *INHA*, *INHBB*, *COL4A1* and *COL4A2* were correlated with earlier onset of PE and earlier delivery of the fetus ( $p < 0.05$ ).

**Conclusion**—These results suggest altered expression of maternal susceptibility genes may play roles in PE development and the course of disease severity.

### Keywords

clinical severity; decidua; gene expression; pre-eclampsia; susceptibility genes

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### Introduction

The key to pregnancy success is the establishment and growth of the placenta. When this is deficient, complications develop that compromise maternal and fetal health. The fundamental origin of pre-eclampsia (PE) lies in the placenta as PE only manifests during pregnancy and can occur in the absence of a fetus (i.e. in a molar pregnancy) [1]. While deficient placentation is characteristic of PE, the underlying causes are unknown [2]. PE has a familial association, with daughters of PE affected women having more than twice the risk of developing PE [3]. Delineating the key processes involved in the establishment of a healthy placenta and how they are altered by genetic variation is central to determining how PE develops.

The familial association of PE is well established. First-degree relatives of women who had a PE pregnancy have a two to five fold increased risk of also having a PE pregnancy [4]. A Norwegian study, examining 1.7 million births from 1967 to 1992, showed that the odds ratio of developing PE in a subsequent pregnancy was 2.2 for mothers with an affected sister [5]. A Swedish study estimated that maternal genetic factors alone accounted for almost half of PE familial aggregation while other factors such as environmental and paternal genetic factors accounted for the rest [6]. PE, like many common human diseases, is thus a complex trait that does not involve simple Mendelian monogenic inheritance [7].

Two approaches are commonly applied to identify causal genetic variation for heritable human diseases: candidate gene-based studies or positional cloning in affected families and/or large population-based cohorts of affected and unaffected individuals. In the candidate gene-based approach, genes are chosen based on the cumulative knowledge of disease pathways. Previous candidate gene studies for PE examined genes involved with

thrombophilia, haemodynamics, endothelial function, inflammation and the immune response, oxidative stress, lipid metabolism, the endocrine system, angiogenesis and placentation [7-9]. Given the low probability of prioritising a functional gene with the incomplete and limited knowledge of the underlying causative mechanisms of PE, no single universally accepted susceptibility gene has been identified.

In the positional cloning approach, underlying disease susceptibility loci are first localised by a genome scan to a potentially large chromosomal region. This localisation is accomplished either by linkage mapping in affected families or by association analysis in large population cohorts of affected and unaffected individuals. The putative susceptibility loci identified are subsequently interrogated for plausible positional candidates that are then subjected to more rigorous genetic analyses. Several groups including our own adopted this positional cloning approach for studying PE. Genome-wide linkage scans were conducted in PE families from Iceland [10], Australia & New Zealand [11-13], the Netherlands [14] and Finland [15]. To date, our studies [12, 13, 16-19] have generated a range of plausible positional candidate genes identified through extensive genome-wide linkage and genome-wide association studies in multiple, well-defined, affected families and unrelated individuals from Australia and New Zealand.

Our genome-wide linkage analyses and fine mapping studies identified significant linkage for the severe PE (SPE) phenotype to chromosomes 2q, 5q and 13q [12, 13, 18]. Bioinformatic prioritisation of positional candidate genes in regions of linkage identified the following genes: on chromosome 2, the activin receptor gene *ACVRI* and the inhibin subunit genes *INHA* and *INHBB*; on chromosome 5, the endoplasmic reticulum aminopeptidase genes *ERAP1* and *ERAP2* and the placental leucine aminopeptidase gene *LNPEP*; and on chromosome 13, the type IV collagen  $\alpha$  chain genes *COL4A1* and *COL4A2*. These genes can be functionally grouped as activin and inhibin signalling components (*ACVRI*, *INHA*, *INHBB*), M1 aminopeptidases (*ERAP1*, *ERAP2*, *LNPEP*) or connective tissue components (*COL4A1*, *COL4A2*).

By employing thorough genetic and bioinformatic analyses, we identified plausible positional candidate maternal susceptibility genes for PE. The aims of this current work were to determine the mRNA expression levels for these candidate genes in decidual tissue collected from third trimester normotensive and SPE pregnancies, and to correlate mRNA expression levels with the clinical severity of SPE.

## Materials and Methods

### Patient Samples

Third trimester *decidua basalis* samples were collected during Caesarean section from n=21 normotensive and n=24 SPE patients as described previously [18]. Normotensive patients underwent Caesarean section due to breech presentation, maternal request or previous history. Samples were classified according to the Australasian Society for the Study of Hypertension in Pregnancy criteria [20, 21], which were previously used to identify the susceptibility genes [12, 13, 18]. Clinical classifications of SPE used meet the current International Society for the Study of Hypertension in Pregnancy guidelines [22] (for

summary of patient clinical characteristics see Table 1). Exclusion criteria consisted of factors known to predispose women to PE such as multiple pregnancies and diabetes. Blood pressures of normotensive patients were recorded as < 140/90mmHg. Patient records were independently verified by a non-treating obstetrician. All tissue samples were verified as decidual by the Royal Women's Hospital pathologists. Written informed consent was obtained from each patient. Research and ethics approval was obtained from the Royal Women's Hospital Research and Ethics Committees. Patient data were analysed for differences in maternal age, gestational age, infant birth weight, infant birth weight percentiles, placental weight, gravidity, parity, and infant sex. We included birth weight percentiles to account for the effect of gestation on infant birth weight [23].

### RNA Extraction, cDNA Synthesis and Real-time Polymerase Chain Reaction (PCR)

Decidual mRNA expression levels of maternal PE susceptibility genes were determined by real-time PCR on patient *decidua basalis* samples. Total RNA was extracted from decidual samples and cDNA was synthesised from the extracted RNA as described previously [18, 24]. Real-time PCR was performed using Applied Biosystems 7500 PCR System (Foster City, CA, USA). The reaction was carried out at 95°C for 10 minutes, followed by 95°C for 15 seconds and 60°C for 1 minute for 40 cycles. Pre-validated TaqMan® Gene Expression Assays (Applied Biosystems, Foster City, CA, USA) with FAM-labelled probes for each gene of interest and VIC-labelled probe for the 18S rRNA housekeeping gene were used. The probes used were: Hs00153836 m1 *ACVRI*, Hs00171410 m1 *INHA*, Hs00173582 m1 *INHBB*, Hs00429970 m1 *ERAPI*, Hs01073631 m1 *ERAP2*, Hs00893646 m1 *LNPEP*, Hs00266237m1 *COL4A1*, Hs01098873m1 *COL4A2* and Eukaryotic *18S rRNA* Endogenous Control (VIC/MGB Probe, Primer Limited) for housekeeping. The average threshold cycle ( $C_T$ ) value difference between FAM and VIC of the pooled normotensive duplicates was used as the calibrator for relative quantification. Relative mRNA expression levels were calculated using the  $2^{-C_T}$  method [25].

### Clinical severity and characteristics analyses

Since the differentially expressed genes belonged to different functional groups, we next looked for relationships of maternal susceptibility gene expression with available patient data in our sample set. We analysed the correlation of mRNA expression levels of the susceptibility genes in the SPE sample set with different parameters of clinical severity and characteristics. The parameters studied were highest recorded systolic and diastolic blood pressures, highest recorded degree of proteinuria, lowest recorded platelet level, infant birth weight percentile, placental weight, gestation at onset of SPE, gestation at delivery of fetus, required treatment with magnesium sulphate, total number of antihypertensive medications prescribed, maternal age, gravidity and parity.

### Immunohistochemistry

Serial 5 µm decidual sections were obtained from frozen decidual tissue of n=4 normotensive and n=4 SPE patients. All washes were performed three times in 1X Phosphate Buffered Saline. Sections were washed to remove embedding media, treated with 3% hydrogen peroxide in dH<sub>2</sub>O to block endogenous peroxidase and washed. Zymed

Laboratories Histostain®-Plus Broad Spectrum Kit (Life Technologies, Carlsbad, CA, USA) was used thereafter following the manufacturer's protocol with the following modifications. Sections were covered with Solution A blocking solution in a humidified chamber for 1 hour at room temperature and then washed. Sections were then incubated in the humidified chamber overnight at 4°C with primary antibodies diluted in 1X Tris Buffered Saline (Supplementary Table 1). After overnight incubation, sections were washed, incubated with Solution B broad spectrum secondary antibody in the humidified chamber for 1 hour at room temperature and washed again. Sections were then incubated with Solution C containing horseradish peroxidase for 1 hour at room temperature and washed. Immunostaining was visualised using AEC substrate solution prepared from Zymed Laboratory AEC Red Substrate Kit (Life Technologies, Carlsbad, CA, USA) according to manufacturer's instructions. Sections were viewed under 200X magnification with an Axioskop 2 light microscope (Zeiss Gruppe, Oberkochen, Germany). Images were captured with a Nikon DXM1200C camera (Nikon Corp., Tokyo, Japan) attached to the microscope.

### Statistical Analyses

Student's *t* test with Welch's Correction was used for comparing parametric data between two groups, Mann-Whitney *U* test was used for comparing non-parametric normalised data between two groups, 2 X 2 contingency table with Fisher's Exact Test was used for comparing categorical data between two groups and Spearman's correlation analysis was used to determine associations between data sets. All data are expressed as mean ± SEM unless stated otherwise. GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical analyses. A value of  $p < 0.05$  was considered statistically significant.

## Results

### Patient Characteristics

Analysis for the  $n=21$  normotensive and  $n=24$  SPE patients showed significant differences in gestational age, birth weight, birth weight percentiles and placental weight, but no significant difference in maternal age, parity, gravidity or infant sex (Table 1).

To account for the significant difference in gestational age between SPE and normotensive patients, we selected a smaller subset of  $n=9$  normotensive and  $n=7$  SPE patients with similar gestational ages. Analysis of this smaller subset showed no significant difference in any patient characteristic analysed (Table 2). Analysis of the residual  $n=17$  SPE patients have been included in Supplementary Table 2.

### Real-time PCR

The results showed significantly increased mRNA expression levels of genes *ACVRI*, *INHBB*, *ERAP1*, *ERAP2*, *LNPEP*, *COL4A1* and *COL4A2* relative to housekeeping *18S rRNA* in SPE compared with normotensive samples, while *INHA* showed no significant difference in the large sample set (Figure 1). Nevertheless, *INHA* showed a trend for increased expression with a *p*-value of 0.052. In the smaller subset, genes *ACVRI*, *ERAP1*, *ERAP2*, *LNPEP* and *COL4A2* retained their significance (Figure 2). Analysis between the

n=7 SPE samples used in the subset and the n=17 residual SPE samples showed no significant difference between the two groups (Supplementary Figure 1).

In the large set, the activin receptor gene *ACVRI* showed a 22.9 fold increase in relative mRNA expression levels. The inhibin gene *INHBB* showed a 3.8 fold increase in relative mRNA expression levels. The aminopeptidase genes, *ERAPI*, *ERAP2* and *LNPEP*, showed 5.3, 11.5 and 21.7 fold increases in relative mRNA expression levels respectively. The collagen genes *COL4A1* and *COL4A2* showed 3.9 and 18.8 fold increases in relative mRNA expression levels respectively. Differential mRNA expression levels of *ACVRI*, *ERAPI*, *ERAP2*, *LNPEP* and *COL4A2* were also detected in the smaller gestationally matched subset, with fold increases of 24.0, 6.1, 22.9, 14.5 and 16.5 in relative mRNA expression levels respectively.

### Clinical severity and characteristics analyses

Correlation analyses of SPE samples in both the large set and smaller subset mostly showed positive associations of increased gene expression with greater disease severity (Table 3). In the large set, decreased *ERAP2* mRNA expression levels were associated with increased systolic blood pressure and a greater number of antihypertensive medications prescribed. Increased *COL4A1* mRNA expression levels were associated with an earlier onset of SPE. Increased *LNPEP* mRNA expression levels were associated with an earlier delivery of the fetus. Increased *COL4A2* mRNA expression levels were associated with increasing parity of the mother. In the subset, increased *COL4A2* mRNA expression levels were associated with an earlier onset of SPE. Increased mRNA expression levels of *INHA*, *INHBB* and *COL4A2* were associated with an earlier delivery of the fetus. Analysis for the residual n=17 SPE samples is presented in Supplementary Table 3.

### Immunohistochemistry

Immunohistochemistry performed on serial decidual sections from normotensive and SPE pregnancies showed decidual localisation of all genes in the decidual stromal cells, the maternal endothelium and the cytotrophoblast cells. Representative sections from a normotensive patient are presented in Figure 3. There were no obvious staining pattern differences between the normotensive and SPE patients (data not shown).

### Discussion

This study examined the mRNA expression levels and protein localisation of candidate maternal PE susceptibility genes in the maternal decidua. We showed differential expression of 7 out of our 8 candidate genes, *ACVRI*, *INHBB*, *ERAPI*, *ERAP2*, *LNPEP*, *COL4A1* and *COL4A2*, in SPE compared with normotensive maternal decidua. The differentially expressed genes belong to several functional groups as described below. We also showed correlations of mRNA expression levels of susceptibility genes with various parameters of clinical severity and characteristics. No difference in protein localisation was qualitatively observed between SPE and normotensive decidua.

All genes significantly differentially expressed in the large set were also differentially expressed in the smaller gestation matched subset with the exception of two genes. The

slight variation in the number of differentially expressed genes between the large set and the smaller subset is probably due to sample size. In the large set, gestational age, placental weight, infant weight and infant weight percentiles were all significantly different between SPE and normotensive patients, but not observed in the smaller subset. As SPE often results in preterm birth, fewer SPE patients go to term and this is thus reflected in the small sample size of the subset. Many published studies have similar differences when using term normotensives as the control comparison group for SPE studies [26-29]. Other studies used a preterm labour group to negate the potential effect of gestational age on gene expression [30-32]. Nevertheless, what triggers preterm labour is a confounder in itself and thus it is debatable as to whether these patients can truly be considered comparable normotensive controls. Additionally, none of the genes in our study were reported to change expression throughout gestation based on a comparative study conducted by Winn *et al.* [33], which compared gene expression between mid-gestation decidual tissues and term decidual tissues. A recent study, which analysed transcription profiles by merging data from different genome-wide platforms, showed that differential decidual gene expression observed in PE can be influenced by gestational age, highlighting the importance of gestational age matching between sample groups [34]. Their analyses did not show the genes examined in this study to be influenced by gestation [34]. All genes *ACVR1*, *ERAP1*, *ERAP2*, *LNPEP* and *COL4A2* differentially expressed in the large set with the exception of *INHBB* and *COL4A1*, were also differentially expressed in the smaller subset, which was gestationally matched, suggesting gestation had no effect on our gene expression and that it is PE state that changes the gene expression.

*ACVR1* and *INHBB* are genes encoding an activin type I receptor and an inhibin  $\beta$  subunit respectively involved in the activin and inhibin signalling pathways [35]. The increased decidual *ACVR1* mRNA expression level in SPE is in contrast to two other activin receptor genes, *ACVR1C* and *ACVR2A*, which were previously found to be decreased in SPE [18]. The increased *ACVR1* expression observed in our study may be a compensatory mechanism to counter-act the decreased expression of *ACVR1C* and *ACVR2A*. The increased *INHBB* expression in SPE decidua is novel. Studies have reported lower or no difference in maternal serum levels of inhibin B, of which *INHBB* is a subunit, in PE as compared normotensive pregnancies [36, 37]. The increased expression observed in our data may be tissue specific and thus not reflected in the circulating maternal blood.

The genes *ERAP1*, *ERAP2* and *LNPEP* that showed significantly increased expression in both sample sets, belong to the M1 family of aminopeptidases, which are involved in regulating blood pressure and parturition through cleaving peptide hormones such as the angiotensins, vasopressin and oxytocin, as well as processing MHC I antigens [38]. *ERAP1* is associated with chronic hypertension and a genetic variant shows reduced enzymatic activity, thus highlighting its role in blood pressure regulation [39, 40]. *ERAP1* is also differentially expressed in essential hypertension [41]. *ERAP2* shares similar functions with *ERAP1* in blood pressure regulation and MHC I antigen presentation [42]. To date, fetal *ERAP2* is associated with PE in an African American population and is altered in first trimester placenta that later develop PE [43, 44]. *LNPEP* activity is lower in PE pregnancies than in normotensive pregnancies [45]. Hence, altered enzymatic activity and

expression may lead to compromised blood pressure regulation and contribute to the hypertension observed in PE. These studies and our work provide strong evidence for a role of the aminopeptidases in PE development.

The expression of collagen type IV  $\alpha$  chain genes *COL4A1* and *COL4A2* are significantly increased in SPE decidua. Increased atypical expression of collagen may lead to poor basement membrane remodelling and result in shallow trophoblast invasion, a key feature of PE pregnancies [12]. The decidua and myometrium that trophoblast cells invade during early pregnancy are rich in collagen type IV, so increased collagen IV expression could alter the extracellular matrix properties [46]. Our data suggest improper collagen expression exists in PE that may contribute to the shallow trophoblast invasion. Additionally, fragments of the non-collagenous domain of *COL4A1* and *COL4A2*, known as arresten and canstatin respectively, have anti-angiogenic effects such as increased apoptosis and decreased proliferation of endothelial cells [47]. In PE, serum levels of these fragments are elevated [48]. Increased collagen expression in the basement membrane would consequently lead to more degradation fragments and the anti-angiogenic effect of these fragments may thus contribute to the endothelial dysfunction observed in PE.

Our data show novel associations of susceptibility gene mRNA expression levels with various clinical parameters of severity and characteristics. Increased expression of the susceptibility genes suggests a compensatory mechanism occurs in PE. Compensation may or may not be sufficient to account for an inherent gene defect, which results in altered gene function such as receptor signalling or enzymatic activity. The associations of decreased expression of blood pressure regulator *ERAP2* with increased systolic blood pressure and number of antihypertensive medications may be due to a genetic defect in *ERAP2* resulting in decreased enzymatic activity. Hence as a compensatory mechanism, there is an increase in the enzyme levels to account for decreased activity. Women with an insufficient increase in enzyme levels are unable to successfully modulate blood pressure, resulting in a higher blood pressure and a greater number of antihypertensive medications required to regulate blood pressure. Nevertheless, the association with systolic blood pressure must take into consideration that most patients were treated with medications, particularly antihypertensives. However, the parameters of the onset of SPE and the delivery of the fetus are unmodifiable by treatments and do reflect the severity of the maternal condition. The negative correlations observed for onset and the delivery of fetus suggest that the increased expression of susceptibility genes may compensate insufficiently, resulting in a correlation with a more severe phenotype with earlier onset and delivery. Increased parity is protective of PE [2], and our data show increased expression of *COL4A2* associates with increased parity, suggesting that increased expression of *COL4A2* is protective and the higher expression observed in our SPE patients may be due to this compensatory mechanism. The exact mechanism that these genes work through in PE remains to be elucidated.

Most PE expression studies have looked at gene expression in the fetal placenta rather than in the maternal decidua [26, 29-31, 49, 50]. Nevertheless, several groups worldwide have conducted several gene expression studies in normotensive and PE decidual samples, mainly employing microarray analyses to identify differentially expressed genes [18, 27, 28, 32]. None of our investigated genes except *INHA* was significantly differentially expressed in

these studies [32]. This is due to two main reasons. Firstly, PE covers a whole spectrum of clinical characteristics from mild to severe PE. The cohorts used in these studies and ours are different. We used a SPE cohort in contrast to a PE cohort used by Løset *et al.* [28] and while Winn *et al.* [32] used a SPE cohort, they used a different set of criteria to define SPE. Additionally, Winn *et al.*'s study [32] uses a preterm labour control group resulting in a different baseline for comparison. Hence, with cohort variability, it is unsurprising that little overlap exists between our study and those in the literature. There is also emerging evidence that PE may be several diseases appearing as one, thus different genes may contribute to the underlying aetiology [51, 52].

Secondly, the candidate genes were identified in the Australian/New Zealand families and individuals through bioinformatics prioritisation of positional candidate genes, and may be specific to these populations. Although possible, this is unlikely as at least one of these genes is also associated with PE in the Norwegian population [17]. Since these genes are susceptibility genes, not every patient would have all genes differentially expressed, and some variation is expected. Interestingly, all but one of our genes showed significantly increased expression in SPE and the one that did not also showed a similar trend to be increased, validating the methods used to identify these genes. Moreover, this expression study was undertaken in patients unrelated to those involved in the previous genetic studies and thus further supports the potential roles of these genes in the underlying aetiology of PE.

## Conclusion

In summary, this study demonstrates differential decidual mRNA expression levels of candidate maternal PE susceptibility genes, identified using a strategy combining quantitative bioinformatics, transcriptional profiling in pregnancy-specific tissue and gene-centric SNP associations with PE. Thus, the gene expression data from our study adds more candidates to the many already identified [53]. The possible consequence of altered expression of these genes on successful placentation and the clinical course of severity remains to be further investigated.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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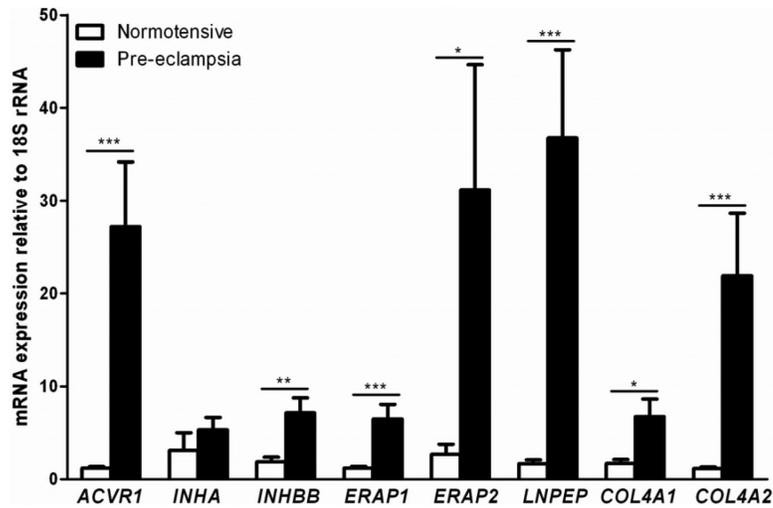
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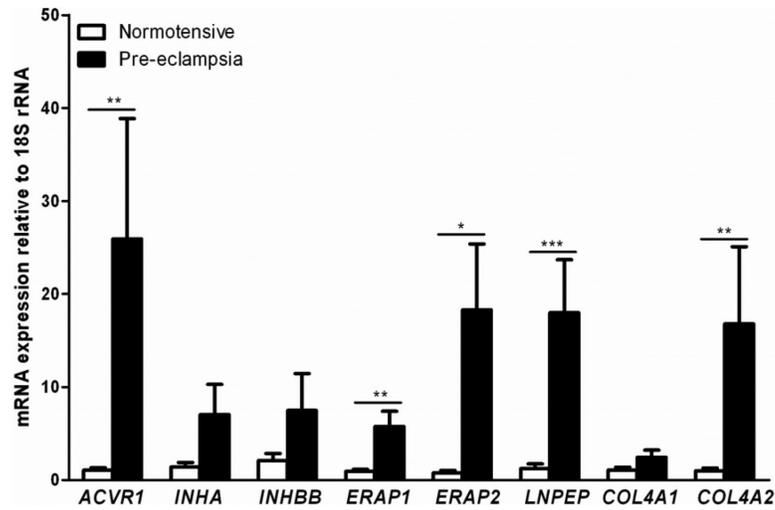
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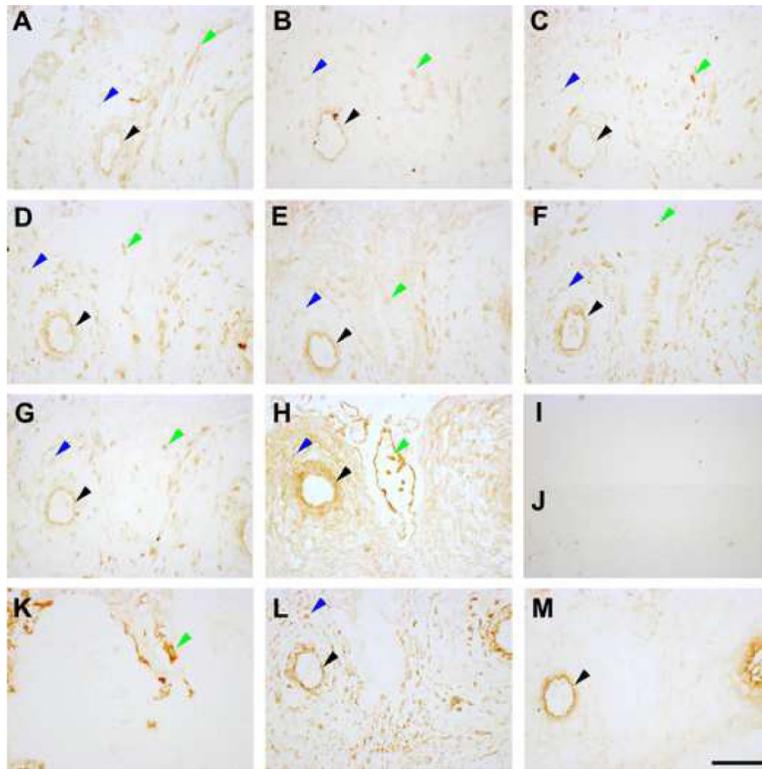


**Figure 1. mRNA expression of maternal pre-eclampsia susceptibility genes: All patient samples**  
 Relative mRNA expression of maternal pre-eclampsia susceptibility genes in human third trimester *decidua basalis* samples from n=21 normotensive and n=24 severe pre-eclamptic pregnancies. Data presented as mean + SEM. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, Mann Whitney U test.



**Figure 2. mRNA expression of maternal pre-eclampsia susceptibility genes: Gestation matched subset**

Relative mRNA expression of maternal pre-eclampsia susceptibility genes in human third trimester *decidua basalis* samples from n=9 normotensive and n=7 severe pre-eclamptic pregnancies. Data presented as mean + SEM. \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, Mann Whitney U test.



**Figure 3. Protein localisation of maternal pre-eclampsia susceptibility genes in the decidua**  
 Decidual sections shown are from a representative patient sample. A: Activin type I receptor (ACVR1) expression. B: Inhibin  $\alpha$  subunit (INHHA) expression. C: Inhibin  $\beta$ B subunit (INHBB) expression. D: Endoplasmic reticulum aminopeptidase 1 (ERAP1) expression. E: Endoplasmic reticulum aminopeptidase 2 (ERAP2) expression. F: Placental leucine aminopeptidase (LNPEP) expression. G: Collagen type IV  $\alpha$ 1 chain (COL4A1) expression. H: Collagen type IV  $\alpha$ 2 chain (COL4A2) expression. I: Non-immune mouse IgG control. J: Non-immune rabbit IgG control. K: Cytokeratin 7 staining. L: Vimentin staining. M: von Willebrand factor staining. Black arrowheads denote endothelium staining, blue arrowheads denote decidual stromal cell staining and green arrowheads denote cytotrophoblast cell staining. Images were taken under 200X magnification. Scale bar denotes 100  $\mu$ m.

**Table 1**

Summary of patient characteristics: All patient samples

Patient characteristics <sup>a</sup>	Normotensive (n=20)	Pre-eclampsia (n=24)	P-value <sup>b</sup>
Maternal age (years)	31.810±0.89	30.29±0.98	0.26
Gestational age (weeks)	38.62±0.43	32.00±0.74	<0.001
Infant birth weight (g)	3193.48±117.43	1662.21±152.30	<0.001
Infant weight percentiles (%) <sup>c</sup>	25-50	10-25	<0.05
Placental weight (g) <sup>d</sup>	583.00±37.79	361.36±22.45	<0.001
Infant sex	11F, 10M	12F, 12M	1.00
Gravidity	16 primi-, 5 multi-	18 primi-, 6 multi-	1.00
Parity	19 primi-, 2 multi-	21 primi-, 3 multi-	1.00
Systolic blood pressure (mmHg) <sup>e</sup>	<140	168.45±2.56	NA
Diastolic blood pressure (mmHg) <sup>e</sup>	<90	105.00±1.92	NA
Antihypertensive treatment(s) <sup>f</sup>	Not given	19	NA
MgSO <sub>4</sub> treatment <sup>f</sup>	Not given	17	NA

NA, not applicable.

<sup>a</sup>Shown is the mean ± SEM unless stated otherwise.

<sup>b</sup>Student's *t* test with Welch's Correction for parametric data and 2 X 2 contingency table with Fisher's Exact Test for categorical data were used.

<sup>c</sup>Data shown as median.

<sup>d</sup>Placental weights for n=3 normotensive and n=3 SPE patients were not recorded.

**Table 2**

Summary of patient characteristics: Smaller subset

Patient characteristics <sup>a</sup>	Normotensive (n=9)	Pre-eclampsia (n=7)	P-value <sup>b</sup>
Maternal age (years)	33.00±1.40	30.57±2.34	0.39
Gestational age (weeks)	37.33±0.80	36.57±0.57	0.45
Infant birth weight (g)	3035.44±230.30	2495.57±260.19	0.14
Infant weight percentiles (%) <sup>c</sup>	50-75	25-50	0.23
Placental weight (g) <sup>d</sup>	592.33±99.36	451.83±52.09	0.25
Infant sex	5F, 4M	3F, 4M	1
Gravidity	6 primi-, 3 multi-	5 primi-, 2 multi-	1
Parity	7 primi-, 2 multi-	6 primi-, 1 multi-	1
Systolic blood pressure (mmHg)	<140	167.57±6.36	NA
Diastolic blood pressure (mmHg)	<90	106.14±5.22	NA
Antihypertensive treatment(s)	Not given	5	NA
MgSO <sub>4</sub> treatment	Not given	5	NA

NA, not applicable.

<sup>a</sup>Shown is the mean ± SEM unless stated otherwise.

<sup>b</sup>Student's *t* test with Welch's Correction for parametric data and 2 X 2 contingency table with Fisher's Exact Test for categorical data.

<sup>c</sup>Data shown as median.

<sup>d</sup>Placental weights for n=3 normotensive and n=1 SPE patients were not recorded.

**Table 3**

Associations of decidual mRNA expression levels to different parameters of clinical severity and characteristics

Clinical severity parameters and characteristics analysed <sup>a</sup>	Associated genes (all SPE samples)	Associated genes (smaller subset SPE samples)
Increasing systolic blood pressure (mmHg)	ERAP2↓	NIL
Increasing diastolic blood pressure (mmHg)	NIL	NIL
Increasing proteinuria (dipstick +)	NIL	NIL
Decreasing level of platelets	NIL	NIL
Greater number of antihypertensive medications	ERAP2↓	NIL
Required MgSO <sub>4</sub> treatment	NIL	NIL
Earlier onset of SPE (weeks)	COL4A1↑	COL4A2↑
Earlier delivery of fetus (weeks)	LNPEP↑	INHA↑, INHBB↑, COL4A2↑
Decreasing infant weight percentile (%)	NIL	NIL
Decreasing placental weight (g)	NIL	NIL
Increasing maternal age (years)	NIL	NIL
Increasing gravidity	NIL	NIL
Increasing parity	COL4A2↑	NIL

<sup>a</sup> Spearman's correlation for correlation of non-parametric data. ↑ denotes increasing expression of gene. ↓ denotes decreasing expression of gene.