The paternal polymorphism rs5370 in the EDN1 gene decreases the risk of preeclampsia

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A B S T R A C T

Objective: To evaluate whether the maternal, paternal or the combined maternal/paternal contribution of SNP rs5370 of the EDN1 gene is associated with preeclampsia and drove its expression in placenta.

Study design: This case-control study included 61 preeclamptic patients and their partners and 49 healthy pregnant women and their partners. The population was sub-divided into three groups: women-only, men-only and combined (women/men). The analysis included genotyping of rs5370 in mothers and fathers and evaluating the expression profile of the EDN1 gene in placenta. Comparisons of categorical variables were performed using chi-square and/or Fisher’s exact tests. The intergroup comparisons were analysed with the Mann-Whitney U test. The association between the polymorphism and the disease was evaluated through multivariate regression analysis. Spearman’s correlation was performed to test the relationship between pre-gestational history and clinical features of the affected patients with EDN1 gene expression.

Results: The analysis of paternal risk factors associated with preeclampsia revealed no differences between groups. A negative association between SNP rs5370 and preeclampsia was found in men group (OR 0.42; CI 95% 0.18–0.94, p = 0.034) but not in women or combined groups. The adjustment for paternal protective factors increased the observed negative association, and the opposite was observed in the presence of paternal risk factors. The expression of the EDN1 gene in the placenta was significantly higher in the group of cases and was not associated with the rs5370 polymorphism.

Conclusion: The paternal rs5370 polymorphism decreases the risk for preeclampsia and is not associated with placental expression of the EDN1 gene.

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1. Introduction

Preeclampsia is a serious pregnancy disorder characterized by de novo hypertension after 20 weeks of gestation and proteinuria (>300 mg in 24 h) [1]; preeclampsia is one of the leading causes of death in pregnancy [2].

Preeclampsia presents with a deficient process of placental implantation, perfusion and ischaemia. The hypoxic placenta releases antiangiogenic and inflammatory/autoimmune factors into the maternal circulation, which activate the endothelium, producing an enhanced synthesis of endothelin 1, a powerful vasoconstrictor [3] encoded by the EDN1 gene.

The infusion in pregnant rats of sFlt1, leads to overexpression of Edn1 in the cortical portion of the kidney but not in the placenta [4]. Hypoxic placenta also release TNF-a [5], which activates the autoantibody Angiotensin II receptor AT1 (AT1-AA) [6] and drives the expression of endothelin 1 in reduced uterine perfusion pressure (RUPP) pregnant rats [7]. Exogenous administration of AT1-AA results in the over-expression of Edn1 mRNA in the cortical portion of the kidney and the placenta, which raises blood pressure [8]. Higher expression of EDN1 has been demonstrated in the placenta of hypoxic rats [9].

No differences in plasma concentrations of endothelin have been reported between preeclamptic and healthy pregnant women...
(HPW) [10]; however, high plasma concentrations of endothelin have been found in preeclamptic patients [11]. This increase was associated with the rs5370 SNP (G/T; Lys198Asn) in the EDN1 gene [12]. Endothelin-1 produces high blood pressure, proteinuria and the suppression of the renin-angiotensin-aldosterone system in preeclampsia [13].

Cnattingius et al. demonstrated the participation of maternal, paternal and foetal genes in preeclampsia [14], whose development depends on the placenta malfunction. One example is the paternal origin of hydatidiform moles that are associated with severe preeclampsia [15].

The role of the father in the aetiopathology of the disease is suggested by primiparity [16], a change in paternity from a previous pregnancy [17], increased interpregnancy intervals [18], the use of barrier contraception [19], conception by intracytoplasmic sperm injection [20], advanced paternal age, paternal family history of cardiovascular events, maternal obesity, racial/ethnic dissimilarity, a short duration of a sexual relationship, primipaternity and long intervals between births [21].

In this study, we aimed to evaluate whether the maternal, the paternal or the combined maternal/paternal contribution of the rs5370 SNP of the EDN1 gene was associated with preeclampsia and drove its expression in the placenta.

2. Materials and methods

2.1. Selection of patients

We conducted a case-control study in which the case group included women affected with preeclampsia and their partners who were compared with HPW and their partners (Table 1). The study population was recruited at the General Hospitals of Durango and Culiacán in Northern Mexico after the approval by the ethics committee of the Ministry of Health at Durango City in accordance with the Code of Ethics of the Declaration of Helsinki. The sample size (n = 144) was calculated to have an odds ratio between 1.0 and 2.0 with 80 percent power, a type 1 error level of 0.05, a minor allele frequency of 0.19 and disease prevalence of 10%.

All women delivered by Caesarean section; indicated in HPW because of iterative cesarean section, cephalopelvic disproportion, prolonged labor and/or foetal distress. To evaluate the participation of the father, information was collected regarding a change of partner, the presence of more than one sexual partner, the time of cohabitation with the same partner, the triggering of preeclampsia in a different woman, and the father being the product of a pregnancy with preeclampsia.

Patients with preeclampsia superimposed on chronic hypertension or concurrent diseases were excluded. The extraction and handling of DNA samples and phenotype measurements were carried out by the same trained personnel. The same diagnosis criteria and procedures were considered for all of the patients at both recruitment centres.

Preeclampsia was defined as mild or severe according to the criteria from the Working Group Report on High Blood Pressure in Pregnancy [1].

2.2. Genotyping analysis

Five millilitres of peripheral blood from each individual were transferred into EDTA supplemented tubes. Genomic DNA was extracted with Qiagen Blood DNA isolation kit (Qiagen, Hilden, Germany). The integrity of the DNA was verified by 1% agarose gel electrophoresis; purity and concentration were verified using spectrophotometry in a Nanodrop 2000c equipment (Thermo Scientific, Suwanee, GA, USA 30024 USA).

Genotyping was carried out with real-time polymerase chain reaction using TaqMan technology with StepOne (Applied Biosystems, Carlsbad, CA, USA) equipment. The final reaction volume was 20 μL using 25 ng of genomic DNA as a template. Thermal cycling conditions were as follows: one cycle of initial denaturing at 95 °C/10 min followed by 42 cycles of denaturing (95 °C/15 s), annealing (60 °C/1 min) and extension (60 °C/30 s). The rs5370 SNP (Lys198Asn) was recognized with the C__598677_1_TaqMan® MGB Probe. The genotyping of samples was performed in triplicate. The specificity of the obtained genotypes was corroborated by automated sequencing in an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA).

2.3. Gene expression analysis

Fresh placentae were obtained after delivery and immediately processed. Twelve small pieces of 0.5 cm² from the maternal side of the placenta were taken and washed with cold 1X PBS. The collected samples (100 mg) were embedded in RNalater® (Ambion®) and stored overnight at 4 °C prior to processing. The RNA was extracted with TRizol® reagent, and the integrity was verified via 1% agarose electrophoresis. After treatment with DNA-free® (Ambion®), the cDNA was synthesized with the High Capacity cDNA Reverse Transcription® (Applied Biosystems®) kit, quantified with a Nanodrop spectrophotometer and stored at −80 °C until use.

The expression of the EDN1 gene in placental samples was evaluated in 24 cases and 24 controls randomly selected from the entire study population. This analysis was carried out in triplicate through semi-quantitative polymerase chain reaction (pPCR) in a StepOne (Applied Biosystems, Carlsbad, CA, USA) instrument with FAM® TaqMan® MGB probes (Applied Biosystems). The probe used for the EDN1 identification was Hs00174961_m1, spanning exons 2 and 3. Beta-2 microglobulin (B2M) was used as the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical and biochemical features of the subjects.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical and biochemical features</td>
<td>Cases n = 61</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>Mean ± Standard error n (%)</td>
</tr>
<tr>
<td>Chronological age (years)</td>
<td>23.9 ± 0.8</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>152.7 ± 2.5</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>98.8 ± 1.5</td>
</tr>
<tr>
<td>Mean arterial pressure (MAP) (mmHg)</td>
<td>111.0 ± 3.6</td>
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<tr>
<td>Pregestational Body Mass Index (BMII) (kg/m²)</td>
<td>25.4 ± 4.1</td>
</tr>
<tr>
<td>Gestational weight gain (kg)</td>
<td>11.3 ± 2.2</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>14.6 ± 2.3</td>
</tr>
<tr>
<td>Leukocytes × 10³/μL</td>
<td>21.7 ± 6.6</td>
</tr>
<tr>
<td>Platelets × 10³/μL</td>
<td>208.1 ± 9.7</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>3 (4.8)</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>7 (11.5)</td>
</tr>
<tr>
<td>Family history of preeclampsia</td>
<td>16 (26.7)</td>
</tr>
<tr>
<td>Personal history of preeclampsia</td>
<td>11 (18.3)</td>
</tr>
<tr>
<td>More than one sexual partner</td>
<td>15 (25.0)</td>
</tr>
<tr>
<td>Pregnancies with a different partner</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td>Consanguinity</td>
<td>3 (4.9)</td>
</tr>
<tr>
<td>Time of cohabitation with the same partner (years)</td>
<td>4.8 ± 4.1</td>
</tr>
<tr>
<td>Primiparity</td>
<td>13 (21.3)</td>
</tr>
<tr>
<td>Newborn birth weights (NBW) (g)</td>
<td>2748.3 ± 106.0</td>
</tr>
</tbody>
</table>

* Mann-Whitney U test.  b Pearson’s X² test.
constitutive gene and was identified with the probe Hs00984230, spanning exons 3 and 4.

The system was validated using Relative Standard Curves for both the EDN1 and B2M genes with efficiencies of 90–100%. The amplification conditions were as follows: 10 min of initial hold at 95 °C followed by 48 cycles of 15 s at 95 °C for denaturing and 1 min at 60 °C for both annealing and extension. Differences in Cycle Threshold values between EDN1 and B2M established the relative expression (RQ) of the EDN1 gene calculated through (2−ΔΔCt) method.

2.4. Statistical analysis

Data are presented as mean ± standard error or proportions. Normality of the data was tested with the Kolmogorov-Smirnov and Shapiro Wilks tests. The frequencies of genotypes were obtained by direct count, and Hardy–Weinberg equilibrium (HWE) was calculated through a chi-square goodness-of-fit statistic. Comparisons of categorical variables were performed using chi-square and/or Fisher’s exact tests. The intergroup comparisons were analysed with the Mann-Whitney U test. The association between polymorphisms and preeclampsia was evaluated using multivariate regression analysis. A Spearman correlation was performed to test the relationship between the pregestational history and the clinical features of the affected patients with EDN1 gene expression. The statistical and genetic analyses were performed using the Statistical Package for Social Sciences (SPSS) software (version 20, SPSS, Inc, Chicago, IL, USA) and SNP Stats (http://bioinfo.iconcologia.net/SNPstats). P-values < 0.05 were considered to be statistically significant.

3. Results

A total of 110 patients and their corresponding partners were enrolled in this study; of these, 61 (55.4%) women with preeclampsia and 49 (44.5%) HPW were allocated to the case and control groups, respectively.

The clinical and biochemical features of the patients are shown in Table 1.

The gestational age was significantly lower in the cases compared to the controls; however, no patients were found with 34 or fewer weeks of gestation. Systolic and diastolic pressures, Mean Arterial Pressure (MAP) and primiparity were significantly higher in the cases. Conversely, the newborn birth weights (NBW) were significantly lower in the case group. The remaining features, were no significantly different between groups.

The case group was further divided according to severity (Table 2). The variables showing differences between groups were systolic and diastolic pressure, MAP, pregestational body mass index (PBMI), AST, platelet count, NBW and deep tendon reflexes.

To conduct the genotyping analysis, the women-only, men-only and combined (female/male) sub-groups were evaluated separately. The analysis of men sub-group demonstrated significant differences between cases and controls in the frequencies of the genotypes GG (P = 0.023) and GT (P = 0.014) but not for the alleles G/T (P = 0.068). In the combined group, significant differences were also observed for genotypes GG (P = 0.045) and GT (P = 0.049). No significant differences between cases and controls were observed for the genotypes or allele frequencies in the women sub-group (Table 3). The sub-groups analysed were all in HWE (P > 0.05). The comparison of allele and genotype frequencies between mild and severe cases showed no significant differences (P > 0.05).

The crude multivariate regression analysis showed a significant negative association between the rs5370 SNP and preeclampsia in the men sub-group (OR 0.42; CI 95% 0.18–0.94, P = 0.034) under a dominant inheritance model. No significant association was observed between cases and controls in the women sub-group (OR 0.98; CI 95% 0.41–2.35, P = 0.97) and the combined group (OR 0.63; CI 95% 0.35–1.14, P = 0.13).

The negative association in the men sub-group increased after adjustment by pregnancies with the same partner (OR 0.27; CI 95% 0.09–0.78, P = 0.013) and sexual intercourse with only one partner (OR 0.32; CI 95% 0.13–0.82, P = 0.015); it was maintained after adjustment by time of cohabitation with the same partner (OR 0.34; 0.14–0.31, P = 0.013), the presence of a family history of preeclampsia (OR 0.42; CI 95% 0.18–0.96, P = 0.038) and
primiparity (OR 0.22; CI 95% 0.08–0.61, P = 0.0033). Conversely, the negative association with the disease was lost after adjustment by personal history of preeclampsia (OR 0.44; CI 95% 0.19–1.02, P = 0.054).

The expression of the EDN1 gene was quantified in 24 placental samples from the cases and controls. A significantly higher level of expression was found in the cases (1.02 ± 0.52) compared to the controls (0.43 ± 0.26; P < 0.001) (Fig. 1). A further analysis of the cases revealed no differences between patients with mild (n = 13) and severe (n = 11) preeclampsia (1.04 ± 0.54 vs 1.01 ± 0.54, P = 0.931). No association was observed between the maternal or paternal genotypes and the EDN1 expression level.

Spearman's correlation coefficient demonstrated that a high expression of endothelin in placenta correlated with early gestational age, higher values of systolic and diastolic pressure, low birth weight and the absence of personal history of preeclampsia (Table 4).

![Fig. 1.](image-url) Comparative expression analysis of EDN1 gene in placental tissue between normoevolutive pregnant women (NEP) and preeclamptic patients. Mann-Whitney U test (P = 0.00 CI 95%).

Table 4: Correlation test for pregestational history and clinical features of the affected patients with EDN1 gene expression.

<table>
<thead>
<tr>
<th>Pregestational history/clinical features</th>
<th>Correlation coefficient r*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronological age (years)</td>
<td>0.047</td>
<td>0.732</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>−0.348</td>
<td>0.010</td>
</tr>
<tr>
<td>Number of pregnancies</td>
<td>0.09</td>
<td>0.503</td>
</tr>
<tr>
<td>Nulliparity</td>
<td>0.08</td>
<td>0.552</td>
</tr>
<tr>
<td>Systolic pressure (mmHg)</td>
<td>0.34</td>
<td>0.013</td>
</tr>
<tr>
<td>Diastolic pressure (mmHg)</td>
<td>0.34</td>
<td>0.014</td>
</tr>
<tr>
<td>Mean arterial pressure (MAP) (mmHg)</td>
<td>0.18</td>
<td>0.208</td>
</tr>
<tr>
<td>Pregestational Body Mass Index (PBMI)</td>
<td>0.085</td>
<td>0.580</td>
</tr>
<tr>
<td>Time of cohabitation with the same partner (years)</td>
<td>0.18</td>
<td>0.189</td>
</tr>
<tr>
<td>More than one sexual partner</td>
<td>0.054</td>
<td>0.703</td>
</tr>
<tr>
<td>Family history of preeclampsia</td>
<td>−0.115</td>
<td>0.390</td>
</tr>
<tr>
<td>Personal history of preeclampsia</td>
<td>−0.320</td>
<td>0.015</td>
</tr>
<tr>
<td>Pregnancies of the same partner</td>
<td>−0.106</td>
<td>0.468</td>
</tr>
<tr>
<td>Platelets × 10^11/L</td>
<td>−0.195</td>
<td>0.230</td>
</tr>
<tr>
<td>Newborn birth weights (NBW) (g)</td>
<td>−0.368</td>
<td>0.007</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>0.167</td>
<td>0.214</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>−0.123</td>
<td>0.361</td>
</tr>
</tbody>
</table>

* Spearman’s correlation coefficient, CI 95%.

In this study, primiparity was significantly more frequent in cases than in controls (P = 0.004), and preeclamptic women were significantly older than healthy women (P = 0.04); however, no subjects were over 40 years of age. Conversely, no significant differences were observed for a personal or family history of preeclampsia, pre-gestational body mass index, and the consumption of alcohol or smoking. All of the subjects in this study were categorized as Mexican mestizo, established by self-perception and the origin of parents and grandparents.

Paternal risk factors for preeclampsia include paternity by a male who had fathered a previous preeclamptic pregnancy with another woman [23] and paternity by a male born from a preeclamptic pregnancy [24]. No man in this study, either from the cases or the controls, admitted to triggering preeclampsia in the pregnancy of a previous partner. One man in the group of cases and two men in the control group reported being the products of preeclamptic pregnancies.

No differences between groups were observed for the presence of more than one sexual partner (P = 1.0). Previous studies have shown that a change in partner increases the risk of preeclampsia [25]; however, this change exerts a predisposing or protective role depending on the presence or absence of preeclampsia in the first pregnancy [26,27].

Preeclampsia is associated with a short time of cohabitation before pregnancy [28]; nevertheless, a shorter time of cohabitation before pregnancy has also been associated with gestational hypertension [29]. In our study, although the time of sexual cohabitation with the same partner was shorter in the group of cases, the difference was not significant (P = 0.328).

Endothelin is considered the final common pathway in the pathophysiology of preeclampsia [30]. In an Australian population, no association was found between the T allele of the rs5370 polymorphism and preeclampsia but rather with a high concentration of serum endothelin and higher systolic pressure [31].

A lack of association between the rs5370 polymorphism and preeclampsia/gestational hypertension was also reported in Poland [32]; paradoxically, this study found an association between the mutated allele T and low blood pressure.

Our results are in agreement with the studies mentioned above because no association between the rs5370 SNP and preeclampsia was observed for the female or the combined sub-groups. Conversely, the evaluation of the male group revealed that the rs5370 SNP confers a reduced risk for preeclampsia (OR 0.42; CI 95% 0.18–0.94, P = 0.034). This negative association remained after adjustment by sexual intercourse with only one partner, a longer time of cohabitation with the same partner and the presence of family history of preeclampsia. This supports the observed decreased risk for preeclampsia associated with the rs5370 polymorphism. The decrease in risk disappeared for those with two or more sexual partners and personal history of preeclampsia.

Interestingly, the reduction in the risk for preeclampsia became larger after adjusting for primiparity (OR 0.22; CI 95% 0.08–0.61, P = 0.0033). This finding was unexpected because primiparity has previously been identified as a risk factor for preeclampsia [22], however, the presence of rs5370 in the EDN1 gene in the father, could regulates the induction of TGFβ in seminal fluid, avoiding a massive exposure in primiparous women.

Few studies have evaluated the paternal contribution to the disease at a molecular level. The Val105/Val105 genotype on the GSTP1 gene in the father and foetus was associated with preeclampsia [33]. The analysis of mother-father-child triads for the MTHFR and FVL genes demonstrated no increased risk in the presence of the child alleles [34]. The frequency of mutations of GSTP1 and eNOS were statistically higher in the control group triads compared to the cases, suggesting a possible protective effect [35]; this study could support our results.

4. Discussion

Our results suggest that the paternal rs5370 SNP is associated with a reduced risk of preeclampsia. Some maternal risk factors have been implicated in the development of preeclampsia [22],
The -604T/C mutation in the KDR gene in the father and foetus, was associated with preeclampsia [36]. Preeclampsia was also associated with the paternal, maternal and foetal C4599A polymorphism on the ACTR2 gene but only in overweight and obese women [37].

In our study, no differences were found for body mass index (BMI) between the cases and controls, so environmental factors other than BMI may be associated with the disease in the general population of Mexico, which has a 74.6% of incidence of overweight and obesity [38].

Aggarwal et al. observed a positive association between the maternal rs5370 polymorphism with preeclampsia, high circulating concentrations of endothelin and higher expression of the EDN1 gene in the placenta of controls compared to preeclamptic patients [11]. Conversely, in this study a lower expression of the EDN1 gene was observed in controls. The earliest gestational age of the controls in the study of Aggarwal et al. (35 ± 3) compared to that of our controls (38.7 ± 0.2), could explain the differences between studies through the decreased expression of EDN1 in normal pregnancy, as it approaches its end.

We found a significantly higher expression of the EDN1 gene in preeclamptic placenta compared with those of HPW (P = 0.000). Supporting our results, Faxén et al. [39] demonstrated a higher placental mRNA expression of EDN1 in preeclamptic vs normal placenta only in the presence of intrauterine growth restriction (IUGR). Similar results were obtained by Dieber-Rotheneder et al. [40] in women diagnosed with early (<34 gestation weeks) vs late onset preeclampsia, with the level of overexpression even greater in the presence of IUGR. In the present study, the overexpression of EDN1 was observed regardless of severity and correlated positively with low birth weight.

Repeated exposure to seminal fluid from the same partner reduces the risk for preeclampsia. Seminal fluid contains high levels of TGFb, which triggers a highly regulated immune response to sustain the pregnancy [41]. TGFb induces endometrial stroma cells to secrete EDN1 mRNA, which acts on the spiral arteries [42,43]. In amnion, TGFb also induces the expression of EDN1 [44], and the last the expression of TGFb [45].

So, it seems reasonable to speculate that high concentrations in seminal fluid, plus the expression of TGFb in decidua and amnion can induce trophoblast migration and endothelial activation through the expression of EDN1.

We did not observe an association between paternal rs5370 polymorphism and placental EDN1 expression, which could be explained by the lack of known functionality of the polymorphism. An alternative mechanism to explain the protective effect of paternal polymorphism rs5370 could be by impairing the interaction with TGF-b. In the father, the presence of rs5370, could decrease the induction of TGF-b in the seminal fluid, folding down the induction of EDN1 in early placenta.

Some limitations of this study deserve mention as follows: an important number of the couples in the group of patients and controls did not accept to participate in the study; thus a large number of patients were not included and the calculated sample size was not achieved. No genotyping was performed in placenta, and serum endothelin was not measured. Thus, further studies are needed to complement our results, such as evaluating the seminal concentrations of TGFb, the polymorphisms of TGFb in men, or the expression levels of the TGFb gene in placenta.

In conclusion, the results of this study suggest that the paternal rs5370 polymorphism in the EDN1 gene decreases the risk for preeclampsia.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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To patients, medic and paramedic personnel of the participant Hospitals.

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