Research Article
Volume 12:7, 2021

Journal of Forensic Research

ISSN: 2157-7145 Open Access

Evaluation of the Association of the Renalase rs10887800 Polymorphism with the Risk of Preeclampsia in Brazilian Women

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Abstract

Despite advances in obstetric medicine, the pathogenesis of preeclampsia (PE) remains poorly understood. It has been suggested that PE results from a state of sympathetic hyperactivity with circulating catecholamines increased in this condition. A new enzyme, called Renalase, has recently been identified exhibiting activity on the metabolism of catecholamine and blood pressure reduction when administered in vivo. Thus, this study evaluated the possible association between the presence of the Renalase gene (RNLS) (rs10887800) polymorphism and mechanisms that control the pathogenesis of PE. This was a cross-sectional, quantitative, case-control study with 94 pregnant women with PE (cases) and 97 normotensive pregnant women (controls). A standardized form was used to collect demographic and clinical data; oral scraping samples were collected, and DNA extraction and subsequent real-time polymerase chain reaction (PCR) were conducted to evaluate the presence of rs10887800. In terms of genotypic distribution and frequency of alleles, no significant association was observed between the rs10887800 polymorphism and development of PE, or with its severe form. However, the GG genotype was associated with a trend of higher risk of PE (GG vs. AG + AA: OR = 2.16, 0.97-4.86, p = 0.05). Hence, the rs10887800 polymorphism could not be determined as a predisposing factor for PE susceptibility or severity in the studied population.

Keywords: Preeclampsia • Renalase • Polymorphism.

Introduction

Preeclampsia (PE) is one of the most prevalent disorders in pregnancy. It corresponds to a specific multisystemic syndrome clinically defined by elevated blood pressure (>140/90 mmHg) and proteinuria that exceeds 300 mg/day, or the presence of clinical and laboratory signs of severity, even without proteinuria, and after the twentieth week of gestation, disappearing after the puerperium [1]. It affects 5 to 8% of pregnant women and is one of the leading causes of maternal and fetal morbidity and mortality [2].Despite the clinical significance of PE, its treatment has not changed substantially over time because the mechanisms underlying its etiopathogenesis remain poorly understood. Thus, a current challenge is to identify biomarkers that detect this syndrome, even before the appearance of its clinical manifestations, favoring alternatives for prevention and treatment.Several etiologies have been proposed to explain the development of PE including placental, immunological, genetic, and environmental causes [6-8]. Recent evidence indicates that it results from sympathetic hyperactivity with levels of circulating catecholamines, such as dopamine, norepinephrine, and adrenaline, increased in this condition.

The degradation of catecholamines through intracellular enzymes, such as catechol - O - methyltransferase, monoamine oxidase - A (MAO-A), and monoamine oxidase B (MAO - B), are involved in regulating blood pressure. In the last decade, researchers have identified a new monoamine oxidase enzyme, which corresponds to a soluble amino oxidase-dependent flavin adenine (FAD) and named it Renalase. Renalase is mainly expressed in the kidney but also found in the heart, small intestine, brain, and skeletal muscle. It has the function of metabolizing catecholamines and their substrates, reducing blood pressure in vivo and suppressing cardiac contractility and heart rate without compensatory changes in the peripheral

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Received: 08 September, 2021; **Accepted:** 22 September, 2021; **Published:** 29 September, 2021.

vascular tone. The Renalase gene is called RNLS, it is located on chromosome 10q23.33 and it is formed by 10 exons and 309,469 base pairs (bp). Its protein consists of 342 amino acids formed by an amino oxidase domain, a FAD-binding region, and a signal peptide. Recent studies have suggested that some single nucleotide polymorphisms (SNPs) in RNLS may be associated with an increased risk of arterial hypertension. Specifically, the RNLS rs10887800 polymorphism has been associated with stroke (stroke), hypertension, type 2 diabetes, end-stage renal disease (ESRD), coronary heart disease, and pregnancy-induced hypertension (PIH). The rs10887800 polymorphism is located at intron 6, close to the exon/intron division in the putative functional region, constituting an intron variant that can affect the mRNA alternative splicing, acting as an RNLS enhancer or regulator of its expression and signaling. Advances in studies in this area have suggested a possible association of the presence of RNLS polymorphisms with mechanisms involved in the PE pathogenesis; however, the literature is still scarce in this subject. Our study is the first in the American continent evaluating the association of the rs10887800 polymorphism with the development of preeclampsia in Brazilian pregnant women.

Methods

Study population

This was a cross-sectional and quantitative case-control study with 94 pregnant women with PE (cases) and 97 normotensive pregnant women (controls). All patients were admitted to the Santa Casa de Misericórdia Teaching Hospital of Sobral. The PE diagnosis was determined according to the International Society for the Study of Hypertension in Pregnancy (ISSHP) [21]. The following criteria were used for PE diagnosis: systolic blood pressure (SBP) and diastolic blood pressure (DBP) equal to or greater than 140/90 mmHg in at least two measurements taken 4 hours apart, and proteinuria above 300 mg for 24 hours. The control group was recruited from the same hospital, consisting of volunteer normotensive pregnant women. The inclusion criteria for those with PE (cases) were: age >18 years; gestational age equal to or greater than 20 weeks; PAS/PAD>140/90 mmHg in at least two measurements taken 4 hours apart; proteinuria above 300 mg for 24 hours, in isolated samples, or with a value equal to or greater than 1+ measured by the semi-quantitative tape method and according to the clinical protocol adopted at this maternity hospital. Severe forms of PE were also included, even in the absence of proteinuria. The inclusion criteria for the control group (controls) were: age>18 years; gestational age equal to or greater than third trimester; not having a history of hypertension or PE; and PAS/PAD≤120/80 mmHg. The exclusion criteria for cases and controls were: multiple pregnancies; the presence of intercurrent diseases such as coagulation disorders, cardiovascular diseases, kidney diseases, autoimmune diseases, liver diseases, and cancer; chronic and gestational hypertension; previous and gestational diabetes mellitus; isolated proteinuria; thrombocytopenia; high levels of transaminases without hypertension; chronic and inflammatory maternal diseases; and pregnancy intrahepatic cholestasis, hepatitis, and stillbirth. Patients with a previous history of PE were also excluded from the control group. PE patients were categorized into two groups based on disease severity, that is, severe PE - defined when presenting one or more of the following criteria: SBP/DBP>160/110 mmHg measured on two occasions with an interval of at least 4 hours, and proteinuria (≥300 mg) in urine sample within 24 hours (it may reach the nephrotic range or present undervalues associated with other severity symptoms), visual disturbances, headache, abdominal pain, high levels of serum creatinine and transaminases, thrombocytopenia, and fetal growth restriction [22-23]. Mild PE was considered when PAS/PAD was between 140-160/90-110 mmHg and proteinuria as <3 g in urine sample within 24 hours, or >1+ on the tape test. The study was approved by the Research Ethics Committee (CEP) of the Federal University of Ceará with the CAAE number of 86263417.2.0000.5054, according to the rules established by Resolution 466/12 of the National Health Council. All patients signed an informed consent form after being informed of the risks and benefits of the study. The handling of biological material was assisted by Resolution CNS No. 441 of May 12, 2011, and was strictly used only for genomic assessment that concerned the objective of this study.

Genomic study

DNA extraction

Samples were obtained by mouth scraping using a cytobrush® brush and stored at –80 o C until further genomic DNA extraction. DNA extraction was performed using the DNA Extract All Reagents Kit (Applied Biosciences®) according to the manufacturer's protocol. Briefly, 50 µL of samples were placed in 1.5 mL microtubes and mixed with 50 µL of lysis buffer. The samples were heated at 95 ºC for 3 minutes and in a pre-heated block and kept at room temperature for 1 minute after the addition of 50 µL of stabilizing buffer solution. The tubes were centrifuged for 1 minute at 10,000 g at room temperature, and the DNA concentration in each sample was quantified using the Qubit dsDNA Assays Broad Range kit (Promega®). These DNA samples were genotyped using real-time PCR (qPCR).

Polymerase chain reaction

The qPCR method was used to determine the presence of the rs10887800 polymorphism in a piece of equipment with a fluorescence reader, coupled to the thermocycler, and capable of measuring light from the amplification reaction. The methodology used TaqMan® fluorophores that correspond to the hybridization probes used to detect specific sequences in DNA amplified by PCR. The genotyping PCR reaction were performed in 96-well plates in a total volume of 10 µL/well. Each reaction was prepared to contain 5 µl of TaqMan GTXpress Mix (2x) (Thermo Fisher®), 0.25 µl of TaqMan SNP Genotyping Assay (40x) (Thermo Scientific®), 2 µl of genomic DNA at 1.5 ng/µl, and 2.75 µl of ultrapure H2O. The thermal cycles were 95 °C for 20 seconds for polymerase activation, followed by 40 cycles of denaturation at 95 °C for 3 seconds and annealing/extension at 60 °C for 90 seconds. The TaqMan GTXpress Mix reagent is composed of AmpliTaq® Fast DNA Polymerase, UP, dNTPs, Tracking Dye, and ROX™ dye. The TaqMan SNP Genotyping Assay reagent (for the detection of rs10887800) consists of one pair of unlabeled primers (final reaction concentration at 900 nM) and the following fluorescent probes: Applied Biosystems™ VIC™ dye - MGB for the A allele and Applied Biosystems™ FAM™ dye - MGB for the G allele (at the final reaction concentration of 200

nM). Bagci et al. published the primer sequences used to amplify the DNA fragments., 2016 ([3]: 5′- CAGGAAAGAAAGAGTTGACAT -3` (Sense) and 5′- AAGTTGTTCCAGCTACTGT -3′ (Antisense)). The genebank number of RNLS. Prepared mixed reactions in tube and plates, with the specific samples, were read in a StepOnePlus Real-Time PCR instrument (Applied Biosystems®) before the performance of the thermal cycles. A post-reading was performed (from which the initial fluorescence values in each sample were discounted), and the data were analyzed using the StepOne™ Software v. 2.3 for allelic discrimination.

Association study

The genotypic distribution involved three forms of variants: homozygous (AA), heterozygous (AG), and homozygous (GG). Each genotype was evaluated under models of genetic heredity (dominant, recessive, and codominant), and the genotypic distribution and allelic frequency were compared among cases and controls.

Statistical analyses

Statistical analyses were performed using the *SigmaPlot*® version 11.0 (SYSTAT. *Software* Inc.). The Shapiro-Wilk test was used to analyze normality in data distribution. The data were presented as mean ± standard error of the mean (SEM), or median with the aid of the t-test (parametric) or Mann-Whitney (non-parametric) test, both from independent samples; values of demographic parameters between PE and control groups, and the biochemical and hematological parameters of women with mild and severe PE were compared. The Pearson X2 test, or the Fisher Exact test, were performed to compare the distribution of genotypes and frequency of alleles in all groups. Logistic regression models were used to assess the association between genotypes and PE.The relative risk approach was assessed by the "odds ratio" with logistic regression considering a 95% confidence interval (CI). A value of $p < 0.05$ was considered to be statistically significant.

Results

Patient characteristics

A total of 191 women were included in the study, 94 pregnant women with PE and 97 healthy pregnant women, who were genotyped for the presence of the rs10887800 polymorphism. The demographic and clinical parameters of subjects are summarized in Table 1. A statistically significant difference was observed between patients with PE and controls in terms of maternal age, gestational age, and body mass index. As expected, the mean systolic and diastolic blood pressure were significantly higher in women with PE when compared to those in the control group ($p = 0.013$ and $p = 0.001$, respectively). This trend was not observed in other analyzed variables.

Variables	Controls (n = 97)	PE Cases (n = 94)	p-value
Maternal age	25.38 ± 0.65	27.31 ± 0.63	0.034*
Gestational age	34.76 ± 0.37	33.45 ± 0.36	0.013*
Body mass index	28.61 ± 0.55	30.81 ± 0.64	0.010*
PAS	113.37 ± 17.42	1 58.15 ± 1.85	0.013*
PAD	74.02 ± 0.86	102.19 ± 1.37	0.001*
Pregnancies (N)	2	2	0.861
Parity (N)	1	1	0.92
Family history of PE (%) (Yes/No)	04/13/86.96	34.83/65.17	0.0004*
Previous history of PE (%) (Yes/No)	0/100	22.47/77.53	0.0000006*

Table 1: General characteristics of patients included in the study.* $p \leq 0.05$ is considered significant (t Student/Mann-Whitney tests).

The biochemical and hematological parameters in the PE group are shown in Table 2 and distributed according to disease severity. A statistically significant difference was observed in platelet values and quantification of uric acid between the group with severe PE compared to the group with mild PE ($p = 0.017$; $p = 0.003$, respectively). No statistical difference was observed between other biochemical and hematological analyzed parameters.

Variables	Mild PE (n = 14)	Severe PE (N=80)	p-value
White blood cells	11,133.57 ± 909.26	11,126.85 ± 346.49	0.994
Platelets	386,642.86 ± 158,642.68	21,7758.67 ± 7,686.10	0.017
Body mass index	28.61 ± 0.55	30.81 ± 0.64	0.01
Hematocrit	4.20 ± 0.29	4.19 ± 0.06	0. 950
Hemoglobin	35.27 ± 0.82	35.53 ± 0.62	0. 861
Creatinine	0.72 ± 0.03	0.80 ± 0.03	0.213
Urea	18.29 ± 1.50	23.97 ± 1.20	0.05
Uric acid	3.99 ± 0.30	5.32 ± 0.18	0.003
Lactic Dehydrogenase	486.86 ± 36.81	644.76 ± 55.31	0.218

Table 2: Biochemical and hematological data in mild and severe PE groups. *$p \leq 0.05$ is considered significant (Student t/Mann-Whitney tests).

Genotypic distribution and allele frequency of the rs10887800 polymorphism

The genotypic distribution and allele frequency of the rs10887800 polymorphism in pregnant women with PE and controls are shown in Table 3.

No significant difference was observed in the rs10887800 polymorphism genotype distribution between the PE and control groups. However, the GG genotype was associated with a trend of higher risk of PE when compared to controls (GG vs. AG + AA: OR = 2.16; CI: 0.97-4.86; $p = 0.05$). No significant difference in allele frequencies was observed between these groups (G vs A: OR = 1.16; CI: 0.71-1.89; $p = 0.61$).

Genotypes (rs10887800)	Controls (n = 97)	PE Cases (n = 94)	OR (95% CI)
AA	28 (28.9%)	27 (28.7%)	(AA vs AG + GG)
AG	44 (45.4%)	54 (57.4%)	(AG vs AA + GG)
GT	25 (27.7%)	13 (13.9%)	(GG vs AG + AA)

Table 3: Distribution of rs10887800 polymorphism genotypes and alleles in the PE and control groups.p ≤ 0.05 is considered significant (Student t/Mann-Whitney tests).

Discussion

PE is a heterogeneous group of disorders in which pregnancy and hypertension coexist and affect maternal and fetal health to varying degrees. Evidence has shown that plasma Renalase is activated in response to higher systolic blood pressure, suggesting that this enzyme plays an important role in the immediate regulation of blood pressure. The recent discovery of this new soluble monoaminoxidase, which plays a relevant role in the degradation of catecholamines in circulation and, subsequently, in the reduction of blood pressure, has been suggested as a new alternative to explain the genesis of PE [17-18]. Current evidence has demonstrated that the concentration of catecholamines is increased in this disorder, especially adrenaline, and therefore, sympathetic activities are increased, suggesting that this could explain the increase in blood pressure this disease.

The rs10887800 polymorphism, the focus of the present study, has been associated with several pathologies such as stroke, regular and pregnancy-induced hypertension, type 2 and gestational diabetes, chronic kidney disease, and heart disease. This polymorphism is located near the exon/intron boundary in a putative functional region that can affect the alternative splicing of messenger RNA, acting as an enhancer or regulator of RNLS gene expression and signaling. The rs10887800 polymorphism may also have a potential role in triggering various diseases and to be a step forward in probing factors that can interact between multifactorial polygenic diseases investigated correlations between RNLS gene polymorphisms (rs10887800, rs2576178, and rs2296545) and the severity of intracranial vascular atherosclerotic stenosis in 212 patients with ischemic stroke by comparing them with 244 healthy controls in a northern China population. These authors identified an association between the GG genotype and G allele and the rs10887800 polymorphism, with disease severity. Stec et al. [26] analyzed the potential association of the rs10887800 and rs2576178 polymorphisms with systemic arterial hypertension in Caucasian patients of Polish origin with end-stage renal disease and identified an association between the G allele of both polymorphisms and an increased risk for systemic arterial hypertension. In the present study, we investigated the potential impact of the rs10887800 polymorphism in the genesis of PE. However, we did not observe statistically significant differences in the rs10887800 polymorphism genotype distribution and allele frequencies between women in the PE and control groups. Nevertheless, the GG genotype was associated with a trend towards a higher risk of PE when compared to controls but without statistical significance. Three studies have assessed the possible association of this polymorphism with PE. In a case-control study of 110 women with PE in a Turkish population, Bagci et al. [3] found a significant association between the presence of the rs10887800 polymorphism G allele and GG genotype and increased risk of PE. Another study conducted with 179 women from Southeast Iran indicated that the rs10887800 and rs2576178 polymorphisms were not associated with PE separately; however, the effect of their combination showed an association between the GG genotype and G allele with a higher risk of PE [4]. The discrepancy between our findings and these studies may be related to the genetic heterogeneity of the studied populations; the effect of these polymorphisms may be different on these diverse studied populations, and these effects might also be affected by geographic variations and epigenetic factors.In a more recent study with 185 Chinese patients with PE, evaluated the association of the rs2296545, rs2576178, and rs10887800 polymorphisms with PE and found a significant association of the first two polymorphisms but no increased risk for PE associated with rs10887800, which corroborates our results. Some of the limitations in our study are the sample size, which is relatively small and resultant from the limiting conditions for sample collection, and the fact that we did not evaluate patients with chronic hypertension complicating the pregnancy, chronic hypertension with overlapping preeclampsia, and gestational hypertension. Regardless, this is the first study carried out on the American continent and, consequently, in Brazil, with the aim of investigating the association between RNLS polymorphisms and the pathogenesis of PE, therefore, making it difficult to compare our results with pre-existing data. Furthermore, because Brazil has a large population size that is significantly mixed, studies with population samples of various races will further the investigation on these potential associations. Our study included a population sample from the Northeast of Brazil, which also does not represent the Brazilian population.

Conclusion

In conclusion, this study, the first to analyze the RNLS rs10887800 polymorphism in a population of Brazilian women with PE, did not demonstrate the association between the presence of this polymorphism and increased risk of PE. However, this result cannot be generalized because a trend of increased risk of PE was observed in women who had a GG genotype. Additional studies with larger sample sizes are recommended to detect the effect of the RNLS rs10887800 polymorphism on the pathophysiology of PE.

Contributions

Mara Suellem de Freitas Moura and José Juvenal Linhares participated in the study conception and design of experiments, statistical analysis, and preparation of the manuscript. Wanneida Fernandes, a clinically experienced obstetrician, assisted with sample collection and clinical information of patients. Emmanuelle Coelho Noronha, Kaio César Simiano Tavares, André Saraiva Leão Marcelo Antunes, and Samara Casemiro Benevides participated in experiments' design and experimental data analyses.

Acknowledgments

We are deeply grateful to all women who participated in the study's sample set, and to the Laboratory of Molecular Biology and Development at the University of Fortaleza led by Professor Adriana Rolim.

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How to cite this article: Linhares Jose Juvenal, Freitas Moura Mara Suellem de, Noronha Emmanuelle Coelho, Benevides Samara Casemiro, et al. "Evaluation of the Association of the Renalase rs10887800 Polymorphism with the Risk of Preeclampsia in Brazilian Women." *J Forensic Res* 12 (2021) : 472