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Polymorphisms in the *IL17A* gene are not involved in the development of preeclampsia in the Brazilian population

Polimorfismos no gene IL17A *não estão envolvidos no desenvolvimento de pré-eclâmpsia na população brasileira*

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ABSTRACT

Introduction: Preeclampsia is defined by the development of hypertension associated with proteinuria after the 20th week of gestation in previously normotensive women. IL17A is a potent inducer of tissue inflammation and polymorphisms in the *IL17A* gene can modulate gene expression and affect the functioning of Th17 cells, strengthening susceptibility to preeclampsia. **Objective:** To investigate the polymorphisms rs4711998 A> G, rs8193036 C> T and rs2275913 A> G in the *IL17A* gene in women with preeclampsia. **Methods:** This is a control case study, composed of 263 women, 89 with preeclampsia and 174 of the control group. The polymorphisms investigated by real time polymerase chain reaction (PCR) allele discrimination technique. The risk of *IL17A* polymorphisms contributing to preeclampsia was assessed by the inheritance model through logistic regression. Statistical power presented 99.5% for association detection. Statistical significance was defined as $p < 0.05$. **Results:** Genotype frequencies as well as multiple logistic regression analysis were not statistically significant for the rs4711998 A>G, rs8193036 C>T and rs2275913 A>G polymorphisms of the *IL17A* gene. No association was found between any haplotypes of the polymorphisms investigated and the risk of developing PE. **Conclusion:** There is no association between the allele frequencies, genotype, inheritance models and haplotypes of the rs4711998 A>G, rs8193036 C>T and rs2275913 A>G polymorphisms of the *IL17A* gene and PE.

Key words: pre-eclampsia; genetic polymorphism; interleukins.

RESUMO

Introdução: *A pré-eclâmpsia (PE) é definida pelo desenvolvimento de hipertensão arterial associada à proteinúria após a semana de gestação em mulheres previamente normotensas. A interleucina 17A (IL17A) é um potente indutor de inflamação tecidual, e polimorfismos no gene* IL17A *podem modular a expressão gênica e afetar o funcionamento das células Th17, contribuindo para a suscetibilidade à PE.* ***Objetivo:*** *Investigar os polimorfismos rs4711998 A>G, rs8193036 C>T e rs2275913 A>G no gene* IL17A *em mulheres com PE.* ***Métodos:*** *Trata-se de um estudo do tipo caso-controle, composto por 263 mulheres, sendo 89 diagnosticadas com PE e 174 do grupo-controle. Os polimorfismos investigados foram avaliados a partir do ácido desoxirribonucleico (DNA) genômico extraído do sangue periférico pela técnica de discriminação alélica por reação em cadeia da polimerase (PCR) em tempo real. O risco de os polimorfismos do gene* IL17A *contribuírem com a PE foi avaliado pelo modelo de herança através da regressão logística. O poder estatístico apresentou 99,5% para a detecção de associação. A significância estatística foi definida como* $p < 0,05$. ***Resultados:*** *As frequências genotípicas, assim como a análise de regressão logística múltipla, não foram estatisticamente significativas para os polimorfismos rs3761549 C>T, rs3761548 A>C e rs2232365 A>G do gene* IL17A. *Não foi observada associação*

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entre nenhum dos haplótipos dos polimorfismos investigados e o risco de desenvolvimento de PE. ***Conclusão:*** *Não há associação entre as frequências alélicas e genotípicas, os modelos de herança e os haplótipos dos polimorfismos rs4711998 A>G, rs8193036 C>T e rs2275913 A>G do gene* IL17A *e a PE.*

Unitermos: *pré-eclâmpsia; polimorfismo genético; interleucinas.*

RESUMEN

Introducción: *La preeclampsia (PE) se define como hipertensión arterial asociada a proteinuria después de la semana 20 de gestación en mujeres anteriormente normotensas. La interleucina 17ª (IL17A) es un inductor potente de inflamación tisular y polimorfismos del gen* IL17A *pueden modular la expresión génica y afectar las funciones de las células Th17, aumentando la susceptibilidad a la PE.* ***Objetivo:*** *Investigar los polimorfismos rs4711998 A>G, rs8193036 C>T y rs2275913 A>G del gen* IL17A *en pacientes con PE.* ***Métodos:*** *Se realizó un estudio de casos y controles, compuesto por 263 mujeres: 89 diagnosticadas con PE y 174 del grupo de control. Se evaluaron los polimorfismos investigados a partir del ácido desoxirribonucleico (ADN) genómico aislado de la sangre periférica por reacción en cadena de la polimerasa (PCR) en tiempo real para discriminación alélica. El riesgo de los polimorfismos del gen IL17A para la PE fue evaluado según el modelo de herencia empleando regresión logística. El poder estadístico presentó 99,5% para la detección de asociación.* ***Resultados:*** *Las frecuencias genotípicas, así como el análisis de regresión logística múltiple, no fueron estadísticamente significativas para los polimorfismos rs3761549 C>T, rs3761548 A>C y rs2232365 A>G del gen* IL17A*. Ningún haplotipo de los polimorfismos investigados mostró asociación con el riesgo de desarrollo de PE.* ***Conclusión:*** *No hay asociación entre las frecuencias alélicas y genotípicas, los modelos de herencia y los haplotipos de los polimorfismos rs4711998 A>G, rs8193036 C>T y rs2275913 A>G del gen* IL17A *y la PE.*

Palabras clave: *preeclampsia; polimorfismo genético; interleucinas.*

INTRODUCTION

Preeclampsia (PE) is defined by the development of hypertension (≥ 140/90 mmHg) associated with proteinuria (≥ 300 mg/24 hours or ≥ 1+ in a reagent strip test) after the 20th week of gestation or after manifestation of signs and symptoms, such as headache, scotomata, abdominal pain, thrombocytopenia (platelets < 100,000/mm^3), elevated liver enzymes (two times baseline), kidney failure (> 1.1 mg/dl), pulmonary edema and convulsions in previously normotensive women[1, 2].

Interleukin 17A (IL17A) is a potent inducer of tissue inflammation secreted by Th17 cells. It activates signaling pathways that induce proliferation, recruiting, activation and migration of neutrophils; it high in the first and third trimesters of pregnancy, favoring the establishment of pregnancy and labor, respectively[3, 4].

IL17A is believed to be involved in the pathogenesis of PE, because studies with animal models have shown that this molecule promotes angiotensin II (Ang-II)-induced hypertension, recruiting leukocytes during hypertensive vascular remodeling[5]. Moreover, high levels of IL17A have been observed in pregnant women with PE, but the involved mechanism is still not fully understood[6].

The gene that encodes IL17A is located in the short arm of chromosome 6 (6p12.1) and is composed of five exons, encoding a protein with 155 amino acids of 17.50 kD[7] molecular weight. Polymorphisms in this gene have already been associated to the pathogenesis of autoimmune and inflammatory diseases[8] and it is believed that they can modulate gene expression, as well as affect the function of Th17 cells, what contributes to susceptibility to PE. However, available data in the literature are scarce and controversial[9, 10], reinforcing the need of studies with this theme in the Brazilian population. Meanwhile, the current work aims at investigating polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G in the *IL17A* gene in women with PE.

MATERIALS AND METHODS

Case study

This is a case-control study, with 263 women treated at the service of gynecology and obstetrics of Hospital de Clínicas da

Universidade Federal do Triângulo Mineiro (UFTM), Uberaba, Minas Gerais, Brazil, between July 2015 and September 2017. This project was approved by the Research Ethics Committee of UFTM (CAAE 44460115.1.0000.5154). All the participants signed a free informed consent.

The study group was formed with 89 women diagnosed with PE according to the criteria of the American College of Obstetricians and Gynecologists (ACOG)[1]. The control group was composed of 174 women with no comorbidities. Participants were just those without history of chronic hypertension, hypothyroidism, diabetes mellitus, gestational diabetes, infectious diseases, autoimmune diseases, multiple pregnancy, and recurrent miscarriages.

Sample genotyping

All participants' 10 ml of whole blood was obtained by venipuncture in blood collection tubes (BD Vacutainer®), with ethylenediaminetetraacetic acid (EDTA). Genomic deoxyribonucleic acid (DNA) extraction was carried out by the phenol-chloroform method[11].

The polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G of the *IL17A* gene were assessed by real time polymerase chain reaction (PCR) allele discrimination technique, using hydrolysis probes TaqMan (ThermoFisher Scientific). PCR reactions had 10 ng DNA, 1.5 µl TaqMan Universal Master Mix (2×), 0.1 µl primers and probes (10×) and ultrapure water for a final volume of 5 µl, including the appropriate negative controls in all assays. Reactions were performed in the instrument StepOnePlus (Applied Biosystems™), under the following conditions: 95°C for 10 minutes and 40 amplification cycles (95°C for 15 seconds and 60°C for 1 minute). For each cycle, the software determined the fluorescent signal from the probes labeled with VICä or FAM.

Statistical analysis

Continuous variables were described as mean ± standard deviation (SD), and the categorical variables were expressed in percentages. Statistical comparisons between two groups were carried out with the use of Mann-Whitney test for quantitative data and Chi-square (χ^2) test for qualitative variables.

In order to assess the risk of polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G of the *IL17A* gene to contribute to PE, a multiple logistic regression analysis was performed, by means of the SNPStats program (available at: http://bioinfo.iconcologia.net/SNPstats_web). In this analysis, the following inheritance models were used: codominant (wild-type homozygous × heterozygous × polymorphic homozygous); dominant (wild-type homozygous × heterozygous× polymorphic homozygous); recessive (polymorphic homozygous × wild-type homozygous + heterozygous). The SNPStats program was also used to infer the haplotypes from the estimated population frequency. Results were presented in odds ratio (OR), with 95% confidence interval (CI). Statistical power presented 99.5% for association detection, using the G Power 3.1 software. Statistical significance was defined as $p < 0.05$.

RESULTS

Participants' characteristics

Participants of this study were 263 women, divided into study group (PE) and control group (C). The PE group was made up of 89 women; and the C group, of 174. Sample characterization can be observed in **Table 1**. Means of maternal age, gestational age, and newborn (NB) weight were significantly lower in group PE (27.1 years, 34.2 weeks, and 2,111.27 grams, respectively). Means of systolic and diastolic blood pressure were statistically higher in group PE (155.8 and 103.3 mmHg, respectively). The number of primigravidae and women with family history of PE was also significantly larger in the PE group (40.4% and 30.3%, respectively).

Genotyping

For polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G in the *IL17A* gene, 260 (173 C and 87 PE), 259 (171 C and 88 PE) and 263 (174 C and 89 PE) samples were analyzed, respectively. The genotypic and allelic frequencies are presented in **Table 2**.

TABLE 1 – Sample characterization

Variables	Group C (n = 174)	Group PE (n = 89)	p
Maternal age (mean ± SD, years)	31.6 ± 7.8	27.1 ± 6.4	< 0.001
Gestational age (mean ± SD, weeks)	39.1 ± 1.3	34.2 ± 3.5	< 0.001
NB weight (mean ± SD, grams)	3,293.8 ± 480.2	2,111.27 ± 855.5	< 0.001
SBP (mean ± SD, mmHg)	114.2 ± 10.4	155.8 ± 22.7	< 0.001
DBP (mean ± SD, mmHg)	75.1 ± 10.3	103.3 ± 14.9	< 0.001
Primigravida, n (%)	26 (14.9%)	36 (40.4%)	< 0.001
Family history of PE, n (%)	15 (8.6%)	27 (30.3%)	< 0.001

SD: standard deviation; NB: newborn; SBP: systolic blood pressure; DBP: diastolic blood pressure; group C: control group; group PE: preeclampsia group. Quantitative variables compared using the Mann-Whitney test; qualitative variables, using the Chi-square test.

TABLE 2 – Distribution of genotypic and allelic frequencies of polymorphisms rs3761549 C>T, rs3761548 A>C and rs2232365 G>A in the *IL17A* gene

Polymorphism	Control *n* (%)	PE *n* (%)	χ^2	*p*
rs4711998				
GG	79 (45.7)	39 (44.8)		
AG	66 (38.1)	35 (40.2)	0.13	0.93
AA	28 (16.2)	13 (15)		
rs8193036				
TT	104 (60.8)	47 (53.4)		
CT	59 (34.5)	31 (35.2)	3.02	0.22
CC	8 (4.7)	10 (11.4)		
rs2275913				
GG	99 (56.9)	59 (66.3)		
AG	65 (37.3)	28 (31.5)	3.02	0.22
AA	10 (5.8)	2 (2.2)		
Allelic frequency				
rs4711998				
A	122 (0.35)	61 (0.35)		
G	224 (0.65)	113 (0.65)		
rs8193036				
C	75 (0.22)	51 (0.29)		
T	267 (0.78)	125 (0.71)		
rs2275913				
A	85 (0.24)	32 (0.18)		
G	263 (0.76)	146 (0.82)		

PE: preeclampsia.

TABLE 3 – Inheritance models of polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G of the *IL17A* gene in the control and PE groups

Model	Genotype	Control *n* (%)	PE *n* (%)	OR (95% CI)	*p*
	rs4711998				
	G/G	79 (45.7)	39 (44.8)	1	
Codominant	A/G	66 (38.1)	35 (40.2)	0.86 (0.48-1.55)	0.8
	A/A	28 (16.2)	13 (14.9)	1.1 (0.5-2.44)	
Dominant	G/G	79 (45.7)	39 (44.8)	1	
	A/G-A/A	94 (54.3)	48 (55.2)	0.92 (0.54-1.59)	0.78
Recessive	G/G-A/G	145 (83.8)	74 (85.1)	1	
	A/A	28 (16.2)	13 (14.9)	1.18 (0.56-2.49)	0.66
	rs8193036				
	T/T	104 (60.8)	47 (53.4)	1	
Codominant	C/T	59 (34.5)	31 (35.2)	0.82 (0.46-1.46)	0.31
	C/C	8 (4.7)	10 (11.4)	0.46 (0.16-1.29)	
Dominant	T/T	104 (60.8)	47 (53.4)	1	
	C/T-C/C	67 (39.2)	41 (46.6)	0.73 (0.43-1.26)	0.26
Recessive	T/T-C/T	163 (95.3)	78 (88.6)	1	
	C/C	8 (4.7)	10 (11.4)	0.5 (0.18-1.35)	0.17
	rs2275913				
	G/G	99 (56.9)	59 (66.3)	1	
Codominant	A/G	65 (37.4)	28 (31.5)	1.31 (0.74-2.32)	0.34
	A/A	10 (5.8)	2 (2.2)	2.55 (0.52-12.49)	
Dominant	G/G	99 (56.9)	59 (66.3)	1	
	A/G-A/A	75 (43.1)	30 (33.7)	1.4 (0.8-2.43)	0.23
Recessive	G/G-A/G	164 (94.2)	87 (97.8)	1	
	A/A	10 (5.8)	2 (2.2)	2.31 (0.48-11.17)	0.26

PE: preeclampsia; OD: odds ratio; CI: confidence interval.

No statistically significant difference was observed between genotypic frequencies and the groups for the investigated polymorphisms ($c^2 = 0.13$; 3.02 and 3.02; $p = 0.93$; 0.22 and 0.22, respectively). Groups PE and control of polymorphism rs8193036 C>T and rs2275913 A>G in the *IL17A* gene are in Hardy Weinberg equilibrium (HWE) (PE: $\chi^2 = 1.83$ and 0.4, and $p = 0.18$ and 0.53; C: $\chi^2 = 0.01$ and 0.02, and $p = 0.92$ and 0.88, respectively). The control group of polymorphism rs4711998 A>G is not in HWE (PE: $\chi^2 = 1.18$ and $p = 0.28$; C: $\chi^2 = 4.67$ and $p = 0.03$).

The multiple logistic regression analysis did not demonstrate statistically significant difference for polymorphisms rs3761549 C>T, rs3761548 A>C and rs2232365 A>G of the *IL17A* gene in any of the inheritance models evaluated (**Table 3**).

Haplotypes

The haplotypes for polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G of the *IL17A* gene are presented in **Table 4**. No association was observed between any of the haplotypes of polymorphisms investigated and the risk of PE development.

TABLE 4 – Haplotypes of the *IL17A* gene

Gene	Haplotype	Control	PE	OR (95% CI)	*p*
IL17A	G-T-G	0.43	0.42	1	-
	A-T-G	0.17	0.16	1.05 (0.52-2.14)	0.89
	G-T-A	0.1	0.09	1.03 (0.37-2.84)	0.95
	A-C-G	0.08	0.13	0.63 (0.31-1.29)	0.2
	G-C-G	0.05	0.09	0.69 (0.28-1.69)	0.41
	A-T-A	0.06	0.02	2.34 (0.34-15.89)	0.39
	G-C-A	0.04	0.03	1.33 (0.36-4.95)	0.67
	A-C-A	0.02	0.02	1.13 (0.25-5.05)	0.88

PE: preeclampsia; OD: odds ratio; CI: confidence interval.

DISCUSSION

Alterations in the immune system have already been associated to the pathogenesis of PE. In this context, IL17A and its potent inflammatory activity seem to play an important role in the etiology of PE[12].

In the present work, mean maternal ages and gestational ages, as well as NB weight were lower in the PE group. This group was also formed by a larger number of primigravidae and women with

family history of PE and presented higher blood pressure levels. Our results agree with those presented in the literature, which indicate that PE is a disease characteristic of the first pregnancy, with increased risk for premature labor and NB below weight, and has a genetic component in its etiology[13-15].

Polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G in the *IL17A* gene are located in the promoting region and can regulate genic expression, playing an important role in the physiopathology processes of several diseases, among which, PE. However, these variants have not been sufficiently exploited in this context[16].

In the present work, no association was observed between polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G in the *IL17A* gene and PE. Similar results related to polymorphism rs2275913 were reported by Wang *et al.* (2015)[10] in a study with 1,031 patients with PE and 1,298 normotensive pregnant women. The authors did not find association between the referred polymorphism and the severity or the onset of symptoms of PE in Chinese women of the Han ethnicity. Anvari *et al.* (2015)[9] investigated the polymorphism rs2275913 in the *IL17A* gene in Iranian women diagnosed with PE – divided according to symptom severity – and 278 healthy pregnant women; no association was found between this polymorphism and severe or mild PE.

Although our results have not associated polymorphism rs2275913 to PE, it seems to modulate the expression of *IL17A* and, consequently, the circulating levels of this cytokine. This was demonstrated by a study performed in Egypt, which observed the association between this polymorphism and the risk of recurrent miscarriages, with genotype AA being related to a higher serum level of IL17A, what demonstrates that the increase of this cytokine is harmful to pregnancy[17].

Up to the moment, there are no available data in the literature about the association between polymorphisms rs4711998 A>G and rs8193036 C>T in the *IL17A* gene and PE, this being the first study to investigate the role of these variants in PE. Nevertheless, they seem to contribute to the uncontrolled inflammation, as observed in studies with coronary artery disease[18], Graves' disease[19], and esophagus cancer[20], which detected association between these polymorphisms and the conditions previously cited.

In the present work, the group C of polymorphism rs4711998 of the *IL17A* gene was not in EHW. Not observing EHW in our study suggests that the assumptions that maintain it are not followed in the control group of this polymorphism and can influence the way alleles are distributed across generations. However, it is not possible to identify precisely which assumption is being violated. Another factor that can influence EHW is the drop-out allelic effect, which occurs when some alleles are insufficiently amplified, leading to an excess of homozygous subjects. This effect apparently does not occur in the present study, because the genotypic frequencies of the control group in these polymorphisms are similar to those of other populations[21].

No association was detected between haplotypes of polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G in the *IL17A* gene and PE. Our results are similar to those found in a study performed in Iran, which did not observe association between haplotypes that contain polymorphism rs2275913 of the *IL17A* gene and the risk of developing PE[9]. However, haplotypes with polymorphisms of the *IL17A* gene have already been associated with autoimmune diseases and miscarriages, as demonstrated by Hammad *et al.* (2016)[22], who associated the haplotype GGA of polymorphisms rs2275913 of the *IL17A* gene and rs763780 and rs2397084 of the *IL17F* gene with juvenile lupus, in Egypt. A study also conducted in Egypt associated haplotype T-A of polymorphisms rs2275913 of the *IL17A* gene and rs763780 of the *IL17F* gene with higher risk of recurrence miscarriages[17].

Although the results about polymorphisms and haplotypes of the *IL17A* gene of the present study are inconsistent in relation to PE, they should not be discarded as allies in identification of alleles predisposing to the disease. Variable results of works about the contribution of certain genes in PE available in the literature can be attributed to factors as genetic diversity of the studied populations and the different employed experimental designs. This, many times, causes the valid associations for certain populations not to be relevant for individuals of other ethnicities, highlighting the complexity of studies involving genetic polymorphisms[17, 23]. Despite this, we believe that in a near future, genetic variants can provide more specific criteria directed to the early detection of PE.

CONCLUSION

We conclude there is no association between the allelic and genotypic frequencies, the inheritance models and the haplotypes of polymorphisms rs4711998 A>G, rs8193036 C>T and rs2275913 A>G of the *IL17A* gene and PE.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

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