Analysis of Polymorphisms in Interleukin-10, Interleukin-6, and Interleukin-1 Receptor Antagonist in Mexican-Mestizo Women with Pre-eclampsia

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Due to the fact that studies seeking associations of polymorphisms in regulatory regions of cytokine genes with pre-eclampsia (PE) have not always been consistent in different population analyses, the aim of this study was to investigate the possible association between rs1800896 of interleukin-10 (*IL-10*), rs1800795 of interleukin-6 (*IL-6*), and the variable number of tandem repeats (VNTR) in intron 2 of interleukin-1 receptor antagonist (*IL-1Ra*), as well as gene–gene interactions between these three polymorphisms with the presence of PE in Mexican-Mestizo women and one Amerindian population from México (Maya). A case–control study was performed where 411 pre-eclamptic cases and 613 controls were genotyped. For the rs1800896 of *IL-10* and rs1800795 of *IL-6*, we used real-time polymerase chain reaction (PCR) allelic discrimination and for the VNTR of *IL-1Ra*, PCR. Allele frequency differences were assessed by Chi-squared test; logistic regression was used to test for associations; a gene–gene interaction was conducted. Genotypic and allelic distribution of the polymorphisms was similar in our population. The estimated of the gene–gene interaction between the polymorphisms did not differ significantly. However, we observed important differences in the distribution of the alleles and genotypes of the three polymorphisms analyzed between Mestiza-Mexicanas and Maya-Mestizo women. In conclusion, we did not find an association between polymorphisms in *IL-10*, *IL-6*, and *IL-1Ra* and PE in Mexican-Mestizo and Maya-Mestizo women. To our knowledge, this is the first time that these three polymorphisms were analyzed together with gene–gene interaction in women with PE.

Introduction

PRE-ECLAMPSIA (PE) IS A public health problem, because it is one of the leading causes of fetal and maternal morbidity and mortality in pregnancy; it is present in 5–7% of pregnant women (Sibai *et al.*, 2005).

The etiology of PE is unknown, but a central pathophysiologic feature of this syndrome is systemic inflammation, which secondarily involves extensive endothelial dysfunction in the maternal vascular system (Redman *et al.*, 1999; Lain and

Roberts, 2002). The levels of proinflammatory molecules and cytokines may play a role in the pathogenesis of this syndrome (Rinehart *et al.*, 1999; Benyo *et al.*, 2001). One of the anti-inflammatory cytokines involved is interleukin-10 (*IL-10*) (Wilczynski *et al.*, 2003; Renaud *et al.*, 2007); furthermore, high levels of inflammatory cytokine, interleukin-6 (*IL-6*), and a naturally occurring competitive inhibitor of *IL-1*, known as interleukin-1 receptor antagonist (*IL-1Ra*), have been detected in serum of women with PE (Greer *et al.*, 1994; Vince *et al.*, 1995; Freeman *et al.*, 2004; Daher *et al.*, 2006).

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Moreover, it has been suggested that polymorphisms in regulatory regions of cytokine genes affect the level of cytokine expression and may be related to predisposition and/or different clinical features or the outcomes of the diseases (Bidwell *et al.*, 2001). Several polymorphisms have been studied in relation with PE, for example, the single-nucleotide polymorphism rs1800896 located in the promoter region of the *IL-10* gene (de Groot *et al.*, 2004; Haggerty *et al.*, 2005; Daher *et al.*, 2006; Kamali-Sarvestani *et al.*, 2006; Vural *et al.*, 2010), the rs1800795 in the promoter region of the *IL-6* gene (Freeman *et al.*, 2004; Saarela *et al.*, 2006), and the variable number of tandem repeats (VNTR) of 86 base pairs (bp) in intron 2 of *IL-1Ra* (Hefler *et al.*, 2001; Lachmeijer *et al.*, 2002; Faisel *et al.*, 2003).

Due to the fact that the results of studies seeking associations of these polymorphisms with PE have not always been consistent in different population analyses (Hefler *et al.*, 2001; Lachmeijer *et al.*, 2002; de Groot *et al.*, 2004; Haggerty *et al.*, 2005; Stonek *et al.*, 2008; Vural *et al.*, 2010), and the Mestizo and Amerindian populations are genetically heterogeneous, the principal aim of this study was to analyze the possible association between rs1800896 of *IL-10*, rs1800795 of *IL-6* and the VNTR in intron 2 of *IL-1Ra*, as well as gene-gene interactions between these three polymorphisms with the presence of PE in Mexican-Mestizo women and one Amerindian population from México (Maya).

Subjects and Methods

The study was approved by the Institute's Human Research Committees. Informed consent was obtained from all patients and controls before participation in the study.

In total, we analyzed 411 unrelated pre-eclamptic women and 613 women without PE of two populations from México: 284 pre-eclamptic women and 350 non-pre-eclamptic women (controls) of Mexican-Mestizo origin and 127 women with PE and 263 pregnant women without PE (controls) of Maya-Mestizo origin.

Only women born in México with a Spanish-derived last name and a family of Mexican ancestors back to the third generation were considered Mexican-Mestizo. In addition, because México City has been a site of massive immigration during the last century receiving inhabitants from all around the country, this group can be considered representative of the overall Mexican population. On the other hand, the Maya-Mestizo women (which are the result of the admixture between the Maya and Spanish population principally) were women with ancestors back to the third generation having at least one Maya surname, and all of whom lived in the state of Yucatán.

A case-control study was performed; for both groups of population, the women admitted with a diagnosis of PE who agreed to participate in the study were recruited and selected consecutively according to their regular visits to the hospital. Pre-eclamptic women of Mexican-Mestizo origin were recruited from the Clinic of Hypertensive Diseases of Pregnancy, Unidad Médica de Alta Especialidad-Hospital de Ginecología y Obstetricia "Luis Castelazo Ayala", Instituto Mexicano del Seguro Social; the control women of the same origin were recruited from the Hospital Regional "Adolfo López Mateos," Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado.

For the women of ethnic Maya-Mestizo, the study was conducted at the Materno-Infantil Hospital of the Secretaría de Salud. This Institution is responsible for providing maternity services to low-income women residing in Mérida, Yucatán, Mexico.

In all cases, upon each visit, a physician or obstetrical nurse measured blood pressure in a seated position via the auscultatory method using a mercury sphygmomanometer. Korotkoff phase V was generally used for defining diastolic blood pressure. PE was defined as the development of hypertension and proteinuria (>300 mg urinary protein in 24 h) in women with no baseline proteinuria. Hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg measured on two consecutive occasions at least 24 h apart (Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy, 2000).

The control group for both populations was comprised of women with uncomplicated pregnancy admitted for natural childbirth or a caesarean section, with normal-length pregnancy, blood pressure $\leq 120/80$ mmHg, and without proteinuria.

Genotyping

Peripheral blood samples were obtained, and genomic DNA was purified as described by Kempter and Grossbadern (1992). Three polymorphisms were studied: the -1082G/A polymorphism in the 5' flanking region of the *IL-10* gene (rs1800896), the -174G/C (polymorphism in the 5' flanking region of the *IL-6* gene (rs1800795), and the VNTR (86 bp-repeat) polymorphisms in intron 2 of *IL-1Ra*.

Genotypes for the VNTR in intron-2 of *IL-1Ra* were determined by polymerase chain reaction (PCR). DNA was amplified by the PCR in 25 μ L of reaction mixture containing 200 ng of genomic DNA, 0.2 mM dNTPs, 2.0 U of Platinum Taq DNA polymerase (Invitrogen, Life Technologies Corporation), and 0.2 μ M of each specific set of intron-2 of *IL-1Ra* primers (the sequence of the primers has been described previously) (Tarlow *et al.*, 1993). Thirty cycles of PCR amplifications were performed in a Thermal Cycler (Multigene II; Labnet International, Inc.). The first cycle was 5 min at 96°C, and except for the last, all cycles were 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C. In the last cycle, the annealing temperature was at 72°C for 5 min. After amplification, PCR products were electrophoresed on 1.2% agarose gels and stained with ethidium bromide to verify the correct size of the expected fragments; the negative control in the PCR having all reagents except DNA. The most common alleles have been termed allele one (A1, four repeats) and allele two (A2, two repeats); also three rare alleles were identified: allele three (A3, three repeats), allele four (A4, five repeats), and allele five (A5, one repeat).

On the other hand, real-time PCR allelic discrimination TaqMan assay (AB) was used for genotyping the -1082G/A of *IL-10* and -174G/C of *IL-6* polymorphisms. All PCRs contained 10 ng of DNA, 5.0 μ L TaqMan Universal Master Mix (AB) (2 $\times$), 0.25 μ L primers and probes (10 $\times$), and water for a final volume of 10 μ L, including the appropriate negative controls in all assays.

In case of the *IL-10* polymorphism, the assay used probes and primers designed by Applied Biosystem assay-on-demand services (assay ID: C___1747360_10), whereas for the

TABLE 1. CLINICAL CHARACTERISTICS OF PRE-ECLAMPTIC WOMEN AND CONTROLS OF TWO MEXICAN POPULATIONS

	Mexican-Mestizo			Maya-Mestizo		
	Cases (N=284)	Controls (N=350)	p-Value	Cases (N=127)	Controls (N=263)	p-Value
Age (years)	28.59±6.3	28.20±6.4	0.46	22.0±5.6	21.59±4.5	0.34
BMI (kg/m ²)	31.57±5.12	29.59±3.80	<0.0001	33.5±5.4	22.5±3.2	<0.0001
SBP (mmHg)	169.17±7.32	113.3±14.32	<0.0001	149.6±11.4	112.8±7.2	<0.0001
DBP (mmHg)	113.68±6.73	77.89±10.10	<0.0001	101.3±8.4	76.7±5.1	<0.0001
Birth weight (g)	1832±1014.6	3069±506.7	<0.0001	2318.6±408.81	3085.8±404.1	<0.0001

Data are means±standard deviation.

BMI, body-mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

–174G/C of *IL-6* polymorphism, the probe and primers were designed by us. Primer sequences were as follows: forward 5'-CGACCTAAGCTGCACTTTTCC-3' and reverse 3'-AGATT GTGCAATGTGACGTCCTT-3'. The probe for each allele is as follows: wild-type VIC 5'-TTGTGTCTTGCGATGCTA-3' and mutant FAM 5'-TTGTGTCTTGCCATGCTA-3'. Real-time PCR was performed on ABI Prism 7500 Fast (Applied Biosystems). Conditions for –1082G/A of *IL-10* and for –174G/C of *IL-6* were 95°C for 10 min and 40 cycles of amplification (92°C for 15 s and 60°C for 1 min). For each cycle, the software determined the fluorescent signal from the VIC- or FAM-labeled probe (Applied Biosystems). Allelic discrimination was performed using specific primers and probes for each allele.

Statistical analysis

Data from the overall patient population in the study were summarized as mean±standard deviation in the case of quantitative variables. Continuous variables were compared by unpaired Student's *t*-test. Statistical analyses were carried out using SPSS v10 (SPSS). Deviations from the Hardy-Weinberg equilibrium were tested using the Chi-squared test. Power calculation of 80%, assuming a 10% difference in genotype percentages, was estimated using the epidemiological data obtained by Duran and Couoh (1999), and the mathematical calculation was performed according to Pértegas Díaz and Fernández (2003).

Allele frequency differences between groups were assessed by Chi-squared tests (<http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl>). In addition, to investigate gene-gene interactions, we applied the generalized multifactor dimensionality reduction (GMDR) methods (Lou *et al.*, 2007).

Results

The clinical characteristics of the pre-eclamptic women and controls of two Mexican populations are shown in Table 1. All cases were women without history of PE. The pre-eclamptic group presented higher diastolic and systolic pressure than the control group ($p<0.001$ for both characteristics). Furthermore, the birth weight was significantly lower in the pre-eclamptic groups than in the women of the control group ($p<0.0001$). Likewise, the body-mass index was significantly different between the pre-eclamptic group and the control group ($p<0.001$). Besides, when we compared the clinical characteristics by a population group of women, we observed

TABLE 2. GENOTYPE AND ALLELE FREQUENCIES OF THE –1082G/A OF *IL-10*, INTRON 2 VARIANTS OF *IL-1RA*, AND –174G/C OF *IL-6* POLYMORPHISMS IN TWO MEXICAN POPULATIONS WITH PRE-ECLAMPSIA

Polymorphism	Cases (n=411)	Controls (n=613)	p-Value
-1082G/A (<i>IL-10</i>)			
Genotype frequencies			
G/G	20 (4.87)	28 (5.43)	0.758
G/A	137 (33.33)	201 (37.43)	
A/A	254 (61.8)	384 (57.14)	
Allele frequency			
G	177 (21.53)	257 (20.96)	
A	645 (78.47)	969 (79.04)	
Intron-2 (<i>IL-1Ra</i>)			
Genotype frequencies			
A1/A1	226 (54.99)	290 (47.31)	0.809
A2/A2	46 (11.19)	74 (12.07)	
A3/A3	2 (0.49)	2 (0.33)	
A1/A2	127 (30.9)	234 (38.17)	
A1/A4	1 (0.24)	6 (0.98)	
A1/A5	0 (0)	1 (0.16)	
A1/A3	4 (0.97)	3 (0.49)	
A2/A3	2 (0.49)	2 (0.33)	
A2/A4	3 (0.73)	1 (0.16)	
Allele frequency			
A1	584 (71.05)	823 (67.13)	
A2	224 (27.25)	386 (31.49)	
A3	10 (1.22)	9 (0.73)	
A4	4 (0.48)	7 (0.57)	
A5	0 (0)	1 (0.08)	
-174G/C (<i>IL-6</i>)			
Genotype frequencies			
G/G	342 (83.21)	522 (85.16)	0.592
G/C	66 (16.06)	83 (13.54)	
C/C	3 (0.73)	8 (1.3)	
Allele frequency			
G	750 (91.24)	1127 (91.93)	
C	72 (8.76)	99 (8.07)	

For the number of individuals (*n*), values in parentheses indicate percentage.

p-Value adjusted for maternal age and body-mass index.

A1, four repeats; A2, two repeats; A3, three repeats; A4, five repeats; A5, one repeat.

IL-10, interleukin-10; *IL-6*, interleukin-6, *IL-1Ra*, interleukin-1 receptor antagonist.

that all Mexican-Mestizo women had severe PE, whereas the Maya-Mestizo women had mild PE.

The Hardy-Weinberg equilibrium test was performed for the polymorphisms under study and showed for the Mexican-Mestizo and Maya-Mestizo populations that the distribution of the observed genotypes did not differ from the expected one, in either the patient or control group ($p > 0.05$). The statistical power of the study was $> 80\%$ at $p < 0.05$ to detect previous associations observed in women with PE.

The genotype and allele frequencies of the three polymorphisms are presented in Tables 2 (for all population) and 3 (for both groups of population). With respect to all population, for the polymorphism $-1082\text{G}/\text{A}$ of *IL-10*, the frequency of the A allele was 78.47% in the PE group and 79.04% among the controls, giving an odds ratio (OR) of 1.035 (95% confidence interval [95% CI]: 0.834–1.2845), while the frequency of the G allele of the $-174\text{G}/\text{C}$ in *IL-6* was 91.24% in the PE group, and 91.93% among the controls, giving an OR of 1.093 (95% CI: 0.796–1.501). Moreover, when we compared the allele and

genotype distribution of both polymorphisms in Mexican-Mestizo versus Maya-Mestizo women, we observed statistically significant differences between these two populations ($p = 0.001$, for both polymorphisms); however, there was no association with PE.

On the other hand, the most frequent alleles of the VNTR in intron 2 of *IL-1Ra* were A1 (four repeats) and A2 (two repeats), being of 71.05% (A1 allele) and 27.25% (allele A2) in the pre-eclamptic group and 67.13% (allele A1) and 31.49% (allele A2) in healthy pregnant women; however, significant differences were not found (Table 2). Additionally, since the genotypes containing A3, A4, and A5 alleles of *IL-1Ra* were rare (1.2% of all population studied), which is in agreement as described by other authors, we excluded from the analysis.

Likewise, when we compared the allele and genotype distribution in Mexican-Mestizo versus Maya-Mestizo women, we observed statistically significant differences between these two populations; the A1 allele (four repeats)

TABLE 3. GENOTYPE AND ALLELE FREQUENCIES OF THE $-1082\text{G}/\text{A}$ OF *IL-10*, INTRON 2 VARIANTS OF *IL-1Ra*, AND $-174\text{G}/\text{C}$ OF *IL-6* POLYMORPHISMS IN MEXICAN-MESTIZO AND MAYA-MESTIZO WOMEN WITH PRE-ECLAMPSIA

	Mexican-Mestizo			Maya-Mestizo		
Polymorphisms	Cases (n=284)	Controls (n=350)	p-Value	Cases (n=127)	Controls (n=263)	p-Value
- 1082G/A (IL-10)						
Genotype frequencies						
G/G	16 (5.63)	19 (5.43)	0.89	4 (3.14)	9 (3.42)	0.539
G/A	107 (37.67)	131 (37.43)		30 (23.63)	70 (26.62)	
A/A	161 (56.7)	200 (57.14)		93 (73.23)	184 (69.96)	
Allele frequency						
G	139 (24.47)	169 (24.14)		38 (14.96)	88 (16.74)	
A	429 (75.53)	531 (75.86)		216 (85.04)	438 (83.26)	
Intron-2 (IL-1Ra)						
Genotype frequencies						
A1/A1	184 (64.79)	207 (59.15)	0.655	42 (33.07)	83 (31.56)	0.779
A2/A2	26 (9.16)	41 (11.72)		20 (15.74)	33 (12.55)	
A3/A3	2 (0.7)	2 (0.57)		0	0	
A1/A2	63 (22.18)	93 (26.58)		64 (50.4)	141 (53.61)	
A1/A3	3 (1.06)	3 (0.85)		1 (0.79)	0	
A1/A4	1 (0.35)	2 (0.57)		0	4 (1.52)	
A1/A5	0	0		0	1 (0.38)	
A2/A3	2 (0.7)	1 (0.28)		0	1 (0.38)	
A2/A4	3 (1.06)	1 (0.28)		0	0	
Allele frequency						
A1	435 (76.59)	512 (73.14)		149 (58.66)	312 (59.31)	
A2	120 (21.13)	177 (25.29)		104 (40.95)	208 (39.54)	
A3	9 (1.58)	8 (1.14)		1 (0.39)	1 (0.19)	
A4	4 (0.7)	3 (0.43)		0	4 (0.77)	
A5	0	0		0	1 (0.19)	
- 174G/C (IL-6)						
Genotype frequencies						
G/G	224 (78.88)	285 (90.11)	0.657	118 (92.91)	237 (90.11)	0.424
G/C	58 (20.42)	59 (9.12)		8 (6.3)	24 (9.12)	
C/C	2 (0.7)	6 (0.77)		1 (0.79)	2 (0.77)	
Allele frequency						
G	506 (89.08)	629 (89.86)		244 (96.06)	498 (94.67)	
C	62 (10.92)	71 (10.14)		10 (3.94)	28 (5.33)	

For the number of individuals (n), values in parentheses indicate percentage.

p-Value adjusted for maternal age and body-mass index.

A1, four repeats; A2, two repeats; A3, three repeats; A4, five repeats; A5, one repeat.

being more frequent in Mexican-Mestizo women ($p=0.001$), and the A2 allele (two repeats) in Maya-Mestizo women ($p=0.001$).

In addition, in multilocus analyses, the GMDR-based analysis did not show a statistically significant interaction for the PE phenotype.

Discussion

In the present study, we analyzed the association of polymorphisms in *IL-10*, *IL-6*, and *IL-1R α* (rs1800896, rs1800795, and VNTR in intron 2, respectively), as well as the gene–gene interactions between polymorphisms of these genes with the presence of PE, in Mexican-Mestizo women and one Amerindian population from México: Maya-Mestizo women. Although this is one of the few studies that have included a significant number of women with PE (411 women) and controls (613 women), resulting in a good statistical power to find out whether Mexican-Mestizo and Mayan-Mestizo women present the same effects as seen in women with PE in other populations (Freeman *et al.*, 2004; Kamali-Sarvestani *et al.*, 2006; Vural *et al.*, 2010), we found no significant associations or interactions between these polymorphisms and the presence of PE in our studied populations.

Several studies suggest that the polymorphism –1082G/A in the promoter region of *IL-10* is associated with PE; furthermore, *in vitro* analysis have shown that this polymorphism modified the levels of *IL-10* production (Warlé *et al.*, 2003; Haggerty *et al.*, 2005; Daher *et al.*, 2006; Kamali-Sarvestani *et al.*, 2006; Vural *et al.*, 2010).

Haggerty *et al.*, (2005) analyzed the –1082G/A polymorphism in white women with PE, and observed that women homozygous for the –1082A allele were more likely to be pre-eclamptic; however, these results were not statistically significantly different. Likewise, Kamali-Sarvestani *et al.* (2006) studied the relationship between the same polymorphism of *IL-10* in Iranian women with PE, and observed contradictory results to those reported by Haggerty *et al.* (2005), since the –1082G allele in a homozygous trait was significantly associated with PE. Furthermore, Stonek *et al.* (2008) analyzed the same polymorphism in Caucasian women, finding that the G allele was more frequent in the pre-eclamptic group than among controls, but these results were not statistically significant. Recently, Vural *et al.* (2010) investigated the same polymorphism in women with PE from Istanbul, finding that the A allele was significantly increased in women who presented this syndrome.

Our results showed that both the analyzed populations present a predominance of the –1082A allele of *IL-10*, finding that our population had the highest percentage of A allele as compared with the different ethnic groups (i.e., Caucasian, Brazilian, Iranian, and Istanbul) (Daher *et al.*, 2006; Kamali-Sarvestani *et al.*, 2006; Stonek *et al.*, 2008; Vural *et al.*, 2010); besides, we found that the GG genotype was present only in 5.15% of the analyzed women, a very low percentage if compared with those populations.

Moreover, no association of the –174G/C polymorphisms of *IL-6* and PE in women of different ethnic groups, such as Caucasian (Freeman *et al.*, 2004; Stonek *et al.*, 2008), Brazilian (Daher *et al.*, 2006), and Istanbul (Vural *et al.*, 2010), was found. Similarly to these studies, in our popu-

lation, the G allele was more frequent, but we did not find association between –174G/C of the *IL-6* gene and presence of PE.

Concerning to the VNTR in intron 2 of *IL-1R α* , there are few reports related to the association of this polymorphism and PE (Hefler *et al.*, 2001; Lachmeijer *et al.*, 2002; Faisel *et al.*, 2003). Hefler *et al.* (2001) analyzed this polymorphism in Hispanic women with PE, finding no association. Likewise, Lachmeijer *et al.* (2002) studied these VNTR in Dutch pre-eclamptic women and found no statistical differences. Similarly, Faisel *et al.* (2003) analyzed this polymorphism in pre-eclamptic women from Finland, noting that the A2 allele (two repeats) was associated with PE. Nevertheless, we did not find this association for the Mexico-Mestizo, nor for Maya-Mestizo population.

In general, PE genetic studies have been focused on the analysis of single polymorphisms in individual genes, delivering inconclusive results; considering the complexity of this syndrome, an alternative approach may need to involve gene–gene interactions. Because none of the analyzed polymorphisms showed association in the population included in our study, we performed an analysis of gene–gene interaction among the three polymorphisms; we did not observe any.

The inconsistencies of the results in different populations regarding the association of the polymorphism –1082G/A in *IL-10*, –174G/C in *IL-6*, and the VNTR intron 2 in *IL-1R α* and PE may be caused by differences among ethnic groups. We previously reported similar differences in other studies related to PE in Maya-Mestizo women (Canto-Cetina *et al.*, 2007; Canto *et al.*, 2008; Díaz-Olgún *et al.*, 2011). Notably, when analyzed separately the –1082A polymorphism of *IL-10* in Mexican-Mestizo versus Maya-Mestizo women, we observed a significant difference in the distribution of the A allele ($p=0.001$). Likewise, for –174G/C of *IL-6*, we found a significant difference in the distribution of the G allele and the GG genotype ($p=0.001$). Finally, for the VNTR in intron 2 of *IL-1R α* , when we compared our two populations, the A1 allele was more frequent in Mexican-Mestizo women and A2 allele in Maya-Mestizo women, observing a significant difference ($p=0.001$).

Taking these results together, we propose that differences in the frequency of alleles and genotypes described in previous reports (Hefler *et al.*, 2001; Lachmeijer *et al.*, 2002; Faisel *et al.*, 2003; Freeman *et al.*, 2004; Stonek *et al.*, 2008; Haggerty *et al.*, 2005; Daher *et al.*, 2006; Kamali-Sarvestani *et al.*, 2006; Saarela *et al.*, 2006; Vural *et al.*, 2010), and in this study, may be due to genetic background, since it is known that Mexican populations are constituted by a genetic admixture among Amerindians, Caucasian, and Black genes. The general pattern has a high Amerindian ancestry, followed by Caucasian and Black ancestry (Lisker *et al.*, 1990).

In addition, it is noteworthy that an important difference between both populations analyzed was that Mexican-Mestizo women had severe PE, and the Maya-Mestizo pre-eclamptic women had mild PE; however, these clinical differences were not associated with the analyzed polymorphisms.

In conclusion, there was no association between the polymorphisms in *IL-10*, *IL-6*, and *IL-1R α* and PE in Mexican-Mestizo and Maya-Mestizo women. To our knowledge, this is the first time that these three polymorphisms

were simultaneously analyzed, as well as gene–gene interaction in pre-eclamptic women. We observed important differences in the distribution of the alleles and genotypes of the three polymorphisms analyzed between Mexican-Mestizo and Maya-Mestizo women. Although this study was based on specific Mexican populations, the clinical relevance of this results is of wide application, as we must also consider that ethnic minorities constitute a growing proportion of the population of developed countries such as the United States (Fowles, 1991); due to migration from different states of Mexico or women of Mexican ethnicity who were born in those countries, a similar trend has been reported for populations from other developing countries.

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Author Disclosure Statement

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