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*J Renin Angiotensin Aldosterone Syst* 2007; 8; 160  
DOI: 10.3317/jraas.2007.026

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# Genetic polymorphisms of the renin-angiotensin system in preterm delivery and premature rupture of membranes

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**Key words:** angiotensin renin system, polymorphism, preterm delivery, premature rupture of membranes, haplotype analysis, linkage disequilibrium

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**Journal of the Renin-Angiotensin-Aldosterone System**

(Including other Peptidergic Systems)

December 2007  
Volume 8  
Number 4

## Abstract

**Introduction.** Premature rupture of membranes (PRM) is a late pregnancy complication commonly associated with preterm delivery (PD). Although several markers related to the renin-angiotensin system (RAS) have been evaluated in certain pregnancy complications, only the angiotensin-converting enzyme (ACE) I/D variant has been studied in PD-PRM. The aim of this survey was to investigate the association of the polymorphisms (angiotensin II type 1 [AT<sub>1</sub>] receptor T174M and M235T, renin G2805A, ACE I/D and AT<sub>1</sub>-receptor A1166C) of the genes of RAS in women with PD-PRM.

**Design.** Deoxyribonucleic acid samples from 89 Mexican Mestizo women with PD and PRM and 224–288 controls were studied. Polymorphisms were analysed by polymerase chain reaction-restricted fragment length polymorphism or sequence specific primer assays.

**Results.** For all *loci*, genotype distribution was in agreement with Hardy-Weinberg expectations in the control group. Significant intergroup difference (case *vs.* control) was seen for angiotensinogen (AGT) M235T polymorphism, with an increased allele M235 in affected cases (50% *vs.* 40% in controls). Analysis of two-*locus* haplotype agrees with an independent segregation of physically unlinked genes. Haplotype AGT 174T-235M was also increased (50% *vs.* 40% in controls).

**Conclusions.** Physically unlinked genes involved in RAS segregate independently. The AGT 174–235 region is associated with PD-PRM in this population.

## Introduction

Premature rupture of membranes (PRM) is a late complication of pregnancy with a prevalence of 8–10%, with demographic variations.<sup>1</sup> It is defined as rupture of the membranes before the onset of labour and is commonly associated with preterm delivery (PD).<sup>2</sup> PRM has a multifactorial etiology and constitutes an important factor for perinatal morbidity and mortality. Some factors such as age, socioeconomic status and ethnicity have been linked to this condition.<sup>3,4</sup> It is also associated with infections, diabetes mellitus, and pre-eclampsia/hypertension, among other factors.<sup>5-8</sup> Some observations suggest the existence of a

genetic predisposition to PRM and PD,<sup>9-14</sup> and several genetic polymorphisms have been proposed as risk factors.<sup>13-19</sup> Previous studies have investigated the association between renin-angiotensin system (RAS) polymorphisms and pregnancy complications, such as stillbirth, abortion, pre-eclampsia, and hypertension.<sup>20-24</sup> Some of these entities are considered risk factors for PD.<sup>25</sup> We have previously reported the association between a genetic marker of RAS, namely the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism and PRM and PD.<sup>8</sup>

All of the RAS elements are expressed in kidneys and in some extra-renal tissues. Since this system is involved in cardiovascular disease,<sup>26-29</sup> some RAS polymorphisms have been studied in complications of pregnancy, such as pre-eclampsia.<sup>30-33</sup>

## Angiotensinogen (AGT)

Fifteen molecular variants are described in both coding and non-coding regions of the AGT *locus*. The most common are the T174M (cytosine replaced by thymine in nucleotide position 521 that results in a threonine-methionine amino acid substitution at position 174) and M235T (thymine by cytosine at nucleotide position 704, and a methionine for threonine substitution at amino acid position 235), located in the 3' region of exon 2. Linkage disequilibrium (LD) has been found between these variants.<sup>34</sup> Genotypes 174M/M and 235T/T (particularly the last one) have a functional effect and are associated with high levels of AGT in plasma.<sup>35</sup> These polymorphisms have been investigated in hypertension<sup>24,36,37</sup> and cardiovascular disease,<sup>38</sup> and pre-eclampsia.<sup>22,30,39</sup>

## Renin (REN)

Renin is the rate-limiting molecule in the production of Angiotensin II (Ang II), the biologically active element of RAS. There are various restricted fragment length polymorphisms (RFLPs) within REN gene: Taq I and Bgl I in the 5' region, Hind III in the 3' region and Hinf I in the first intron.<sup>40</sup> Bgl I is the most studied variant. It has been proposed that allele Bgl I+ (or G) is a risk factor for hypertension, heart failure and diabetes mellitus.<sup>41-44</sup>

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Accepted for publication  
12th June 2007

JRAAS 2007;8:160-8

## Journal of the Renin-Angiotensin-Aldosterone System

(Including other Peptidergic Systems)

December 2007  
Volume 8  
Number 4

### ACE

Fifty-five per cent of the interindividual variability in the plasma concentration of ACE is determined by an I/D polymorphism located close to the 3' end at intron 16. This polymorphism is defined by the presence or absence of a 287 bp stretch of repetitive Alu sequences,<sup>45</sup> with allele D being associated in a co-dominant form with ACE levels (D/D > I/D > I/I).<sup>46</sup> Although a 'functional' polymorphism characteristic has been proposed, it is possible that the effect is due to another truly functional mutation in strong LD with ACE I/D.<sup>47</sup> Similarly, the D/D genotype has been associated with cardiovascular and thrombotic diseases,<sup>26,48</sup> including thrombosis in pregnancy<sup>31,49</sup> and pre-eclampsia.<sup>23,32,50</sup> In a previous study from our group, the ACE I/D polymorphism was studied in women with PD and a high prevalence of the D allele was observed in patients with PRM, with an OR = 5.31, and a CI 95% = 1.25–25.98.<sup>18</sup>

### Angiotensin II type 1 (AT<sub>1</sub>) receptor

Ang II acts mainly through a receptor type 1 called AT<sub>1</sub>-receptor. Various polymorphisms have been detected in this receptor, the most common being an adenine/cytosine base substitution (A/C) in position 1166 within the untranslated 3' region. Since this is a non-functional variant, it is suggested that associations result from LD with other functional genomic regions.<sup>51</sup> The C allele is considered a risk factor for essential hypertension, pre-eclampsia,<sup>33,52</sup> and gestational hypertension.<sup>24,53</sup> A potential epistatic interaction possibly exists between ACE D/D and AT<sub>1</sub>-receptor A/C or C/C, genotypes that produces a synergistic effect in some diseases.<sup>54</sup>

The goal of this study was to investigate RAS polymorphisms (AGT T174M and M235T, REN G2805A, ACE I/D and AT<sub>1</sub>-receptor A1166C) in patients with PD-PRM, and to analyse haplotype distribution and LD between these polymorphisms.

## Materials and methods

### Populations studied

In this case-control study, data from cases were obtained from 89 nonconsecutive Mexican Mestizo women with PD-PRM (22–36-weeks of gestation from the last menstrual period, with PRM of 24-hours or more, age range 16–40 years), resolved either by vaginal delivery or caesarean section, who attended the Gynecology-Obstetrics Hospital, National Western Medical Center, and General Hospital No. 45, Mexican Institute of Social Security, in Guadalajara, Mexico, between October 1999 and January 2003. Women whose PD was induced were excluded.

Individuals from the control group (CON) came from two sources: one group drawn from the general population and another composed of

women with normal labour. Since genetic data comparison between these groups resulted in no statistical significance (0.25 *p* 1.0), they were pooled. The general population controls included at least 157 unrelated and reportedly healthy adults living in Guadalajara. Male:female ratio in this group was 1:1, and their age range 18–65 years. The group of women with normal labour consisted of 67 women with at least one normal labour and without a history of PD or PRM, with an age range 15–38 years. Sample size was different in the case of the ACE polymorphism because data were pooled with those from a previous study.<sup>18</sup> Every person agreed voluntarily to participate in the study and signed an informed consent form. The protocol was approved by the local ethics committee.

### Data for the PD-PRM group

Gestational age and the method of delivery were obtained from medical records. The presence or absence of the following variables was also noted: previous premature delivery, infection (specifically genitourinary), PRM, hypertensive disorders (pre-eclampsia/eclampsia/hypertension), and type 2 or gestational diabetes mellitus.

### Laboratory typing

In all cases, DNA was extracted from peripheral blood.<sup>55</sup> Single nucleotide polymorphisms AGT: T174M, M235T;<sup>56</sup> REN: G2805A (unpublished data 2005), ACE: I/D,<sup>56</sup> and AT<sub>1</sub>-receptor A1166C<sup>57</sup> were analysed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or PCR-sequence specific primers (PCR-SSP), electrophoresis in acrylamide gels, and silver staining. Sample size varies per polymorphism due to PCR failure in some individuals.

### Data analysis

For sample size calculations, we considered 86 cases and 172 controls (172 and 344 chromosomes, respectively) as adequate to identify as significant an OR of 4.0 or more when the frequency of exposure to the risk factor equals 5%. This sample size is also adequate to identify an 8% difference in common allele (> 5%) frequencies with a level of significance of 0.05 and a power of 0.80.

For intragroup analysis, a minimum of 10 individuals (20 chromosomes) per category were studied. Genetic comparisons were made between both categories per variable. This sample size is adequate to identify as significant a difference of 15% or greater in the frequency of common alleles, with significance of 0.05 and a power of 0.80.<sup>58</sup>

Allele frequencies were calculated by genotype counting.<sup>59</sup> Observed genotype proportions were compared with Hardy-Weinberg expectations (HWE) by exact and  $\chi^2$  tests. Inter (PD-PRM *vs.* CON groups) and intragroup (within PD-PRM

**Table 1**Genotype and allele frequencies by *locus* in control and case groups, counts (%).

<i>Locus</i>	Group	Genotype			Allele	
		T/T	T/M	M/M	T	M
<b>AGT T174M</b>	CON (n=224)	177 (79)	45 (20)	2 (1)	399 (89)	49 (11)
	PRM (n=89)	71 (80)	18 (20)	0 (0)	160 (90)	18 (10)
<b>*AGT M235T</b>	CON (n=224)	75 (33)	118 (53)	31 (14)	268 (60)	180 (40)
	PRM (n=89)	16 (18)	57 (64)	16 (18)	89 (50)	89 (50)
<b>REN G2805A</b>	CON (n=224)	136 (61)	75 (33)	13 (6)	347 (77)	101 (23)
	PRM (n=86)	47 (55)	33 (38)	6 (7)	127 (74)	45(26)
<b>ACE I/D</b>	CON (n=288)	74 (26)	151 (52)	63 (22)	299 (52)	277 (48)
	PRM (n=89)	19 (21)	52 (59)	18 (20)	90 (51)	88 (49)
<b>AT<sub>1</sub>-receptor A1166C</b>	CON (n=224)	121 (54)	83 (37)	20 (9)	325 (73)	123 (27)
	PRM (n=86)	48 (56)	33 (38)	5 (6)	129 (75)	43 (25)

**Key:** AGTM235T intergroup distribution differences, exact test p values: \*p=0.02 for genotype (all three), p=0.03 for allele (M increased in group PRM), and p=0.005 for phenotype M (genotypes T/M+M/M), increased in group PRM); p>0.05 for other polymorphism comparisons. Definition of alleles: T = threonine; M = methionine; A = adenine; G = guanine; I = Insertion; D = deletion; C = Cytosine.

group) genotype, phenotype and allele distributions were compared using an interactive computer program also based on  $\chi^2$  and exact tests. Crude OR was estimated for each risk factor. All variables with p values < 0.25 were included in multivariate analysis. Interaction between variables was evaluated by adding to the models a new variable formed by the multiplication in pairs of those statistically significant ( $p < 0.05$ ), and no interactions were found. The analysis of all possible confounding variables was applied to models, including on the model all those variables which differ from their estimates by more than 10%. Again, no confounding effect was identified. Goodness-of-fit was evaluated by the test of Hosmer and Lemeshow,<sup>60</sup> and odds ratios were calculated by logistic regression with version 10 SPSS (SPSS, Chicago, IL) software package.

### Estimation of haplotype frequencies

Two-*locus* haplotype distribution and LD were estimated in cases and in controls independently. The estimation was carried out with the computer program Arlequin ver. 2.000,<sup>61</sup> based on the expectation-maximisation algorithm by the maximum likelihood method. The comparisons of haplotype frequencies were performed by exact and  $\chi^2$  tests.

The crude LD was estimated from the differences among the observed and expected frequencies by the products of the gene frequencies. The LD ( $D'$  or  $D$  normalised) was calculated according to the literature<sup>62</sup> as  $D' = D/D_{\max}$ ; where  $D = x-pq$  ( $x$  is the observed haplotype frequency and  $pq$  is the expected haplotype frequency); and  $D_{\max} =$

## Results

### Intergroup analysis

Genotype distributions for all the *loci* in the CON group were in agreement with HWE. The  $\chi^2$  (one degree of freedom) and p values from differences among observed and expected proportions were: AGT T174M:0.22, 1.00; AGT M235T:2.05, 0.17; REN A2805G:0.38, 0.57; ACE I/D: 0.72, 0.48; AT<sub>1</sub>-receptor A1166C:1.09, 0.31. The genotype, allele and phenotype frequencies by *locus* are shown in table 1.

The intergroup comparisons showed significant difference only for the AGT M235T polymorphic marker, in which an increase of M allele was found in the group of women with PD-PRM (50% *vs.* 40% in controls,  $p=0.027$ ). In this site, a significant difference in the genotype distribution was also observed, namely an excess of M/T heterozygotes in the PD-PRM group (64% *vs.* 53% in controls,  $p=0.019$ ). The difference in the phenotype distribution was also statistically significant, with the AGT 235M phenotype (genotypes M/M + M/T) increased in patients with PD-PRM (82% *vs.* 67% in controls,  $p < 0.01$ ). The OR for the 235M phenotype was 2.30 (IC<sub>95</sub> 1.21–4.42).

### Intragroup analysis (case group only)

The women with a history of PD and PRM were further stratified according to the variable considered (table 2). Only the method of delivery and the presence or absence of hypertensive disorders resulted in a significant difference. The

**Table 2**  
Variables of the women group with preterm delivery and premature rupture of membranes and their frequencies.

Characteristic	Observed frequency (%)
Gestational age (weeks):	
32–36	31 (35)
28–31	45 (50)
< 28	13 (15)
Method of delivery:	
vaginal labour	40 (45)
caesarean section	49 (55)
Previous PD and PRM	22 (25)
Diabetes mellitus	3 (3)
Infection (specifically genitourinary)	34 (38)
Hypertensive disorders	11 (12)

**Key:** PD = preterm delivery; PRM = premature rupture of membranes.

genotype and allele frequencies by subgroup are presented in table 3.

- Method of delivery. In the vaginal labour subgroup, there was an increment of the D allele of ACE (61% *vs.* 40% in women with caesarean section,  $p < 0.01$ ), as well as of the D/D genotype (32% *vs.* 10% in caesarean section,  $p < 0.02$ ). The D phenotype that includes genotypes D/D and D/I also showed a statistically significant difference (90% in women with vaginal labour *vs.* 69% in those with caesarean section,  $p < 0.02$ ). The OR for D phenotype was 3.97 (IC<sub>95</sub> 1.20–3.16).
- Hypertensive Disorders. Although a significant difference was not observed in genotype, the C allele of AT<sub>1</sub>-receptor polymorphism was increased in the group with hypertensive disorders (45% *vs.* 22% in the group without hypertensive disorders), as well as C

phenotype (T/C + C/C; 80% *vs.* 40% of absence of hypertensive disorders); with p values of 0.045 and 0.015, respectively. The OR for the C phenotype was of 6.13 (IC<sub>95</sub> 1.09–45.16).

**Two-locus haplotype analysis in physically unlinked genes**

The estimation of two-locus haplotype (pairs of *loci*) frequencies (four possible haplotypes by pairs) was carried out for the three polymorphisms of physically independent *loci* AGT M235T, REN G2805A and AT<sub>1</sub>-receptor A1166C. The ACE gene was not included in these comparisons because the control population for this polymorphism was not exactly the same as that of other genes. With respect to the AGT gene, only the most informative polymorphism (M235T) was included in the analysis due to the physical proximity (strong LD) of sites within this gene region. The observed (estimated by maximum likelihood) and expected (from component allele frequency) frequencies and the obtained LD values are shown in tables 4 and 5.

REN G2805A – AGT M235T haplotype distribution comparison between cases and controls yielded a borderline significance ( $p = 0.047$ ), with an increase of REN G-AGT M (GM) haplotype in the patients with PD-PRM (17% *vs.* 9% in controls,  $p < 0.01$ ), and an OR for this haplotype of 1.91 (IC<sub>95</sub> 1.11–3.27).

**Haplotype and linkage disequilibrium analysis within the AGT gene**

The haplotype analysis of linked sites was made for codons 174 and 235 of the AGT gene. Four possible haplotypes 174–235 were defined: (TT, TM, MT, MM). The observed and expected frequencies and the LD values are shown in table 6.

The haplotype frequency comparison between cases and controls showed a different intergroup

**Table 3**  
Genotype and allele frequencies by *locus* and subgroup in cases group, counts (%).

Locus	Subgroup	Genotype			Allele	
		I/I	I/D	D/D	I	D
ACE I/D	CS (n=49)	15 (31)	29 (59)	5 (10)	59 (60)	39 (40)
	VL (n=40)	4 (10)	23 (58)	13 (32)	31 (39)	49 (61)
AT <sub>1</sub> -receptor A1166C	HD + (n=10)	A/A	A/C	C/C	A	C
	HD - (n=76)	2 (20)	7 (70)	1 (10)	11 (55)	9 (45)
		46 (60)	26 (34)	4 (6)	118 (78)	34 (22)

**Key:** ACE D exact test p values:  $p = 0.02$  for genotype (all three), and for phenotype D (genotypes I/D+D/D, increased in subgroup VL);  $p < 0.01$  for allele (D increased in subgroup VL); AT<sub>1</sub>-receptor 1166C exact test p values:  $p = 0.045$  for allele (C increased in subgroup HD+); AT<sub>1</sub>-receptor = angiotensin type 1 receptor; ACE = angiotensin-converting enzyme; A = adenine; D = deletion; C = cytosine; I = insertion; CS = cesarean section; VL = vaginal labour; HD = hypertensive disorders presence (+) or absence (-).

**Table 4**Two-locus haplotype frequencies and linkage disequilibrium in three *loci* in control group (n=224), counts (%).

Haplotype	Observed frequency	Expected frequency	$\chi^2$	p	D'
<b>REN-AGT</b>					
A-T	210 (47)	208 (46)	0.31	0.96	0.060
A-M	137 (31)	139 (31)			
G-T	58(13)	60 (13)			
G-M	43 (9)	41 (9)			
<b>AGT-AT<sub>1</sub>-receptor</b>					
T-A	200 (45)	194 (43)	1.45	0.78	0.113
T-C	68 (15)	74 (17)			
M-A	125 (28)	131 (29)			
M-C	55 (12)	49 (11)			
<b>REN-AT<sub>1</sub>-receptor</b>					
A-A	249 (56)	252 (56)	0.48	0.92	0.098
A-C	98 (22)	95 (21)			
G-A	76 (17)	73 (16)			
G-C	25 (5)	28 (6)			

**Key:** Alleles as in table 1. AGT = angiotensinogen; AT<sub>1</sub>-receptor = angiotensin type 1 receptor; M = methionine; A = adenine; G = guanine; C = cytosine; D' = normalised linkage disequilibrium; REN = renin; T = threonine.

**Table 5**Two-locus haplotype frequencies and linkage disequilibrium in three *loci* in cases group (n=89), counts (%).

Haplotype	Observed frequency	Expected frequency	$\chi^2$	p	D'
<b>REN-AGT</b>					
A-T	72 (42)	65 (38)	5.94	0.025	0.320
A-M	55 (32)	62 (36)			
G-T	16 (9)	23 (13)			
G-M	29 (17)	22 (13)			
<b>AGT-AT<sub>1</sub>-receptor</b>					
T-A	64 (38)	65 (38)	1.35	0.74	0.161
T-C	25 (14)	62 (36)			
M-A	56 (38)	23 (13)			
M-C	17 (10)	22 (13)			
<b>REN-AT<sub>1</sub>-receptor</b>					
A-A	91 (53)	95 (55)	2.91	0.40	0.378
A-C	36 (21)	32 (19)			
G-A	38 (22)	34 (20)			
G-C	7 (4)	11 (6)			

**Key:** Alleles as in table 1. AGT = angiotensinogen; AT<sub>1</sub>-receptor = angiotensin type 1 receptor; D' = normalised linkage disequilibrium; T = threonine; M = methionine; A = adenine; G = guanine; C = cytosine; REN = renin.

distribution ( $p < 0.0005$ ). Considering individual haplotypes, an increment of the 174T-235M haplotype was observed in patients with PD-PRM (50% *vs.* 40% in controls,  $p = 0.02$ ). The OR for this haplotype was of 1.52 (IC<sub>95</sub> 1.05–2.18).

## Discussion

### Intergroup and intragroup comparisons

With respect to the AGT polymorphisms, previous studies have proposed an association between the 174T/T and 235T/T genotypes.<sup>63</sup> The latter implies a functional effect since a relationship to greater levels of circulating AGT has been established.<sup>35</sup> It has also been proposed<sup>35</sup> that the 235T allele increases levels

of AGT.<sup>64</sup> Nevertheless, a convincing biological explanation for this association is still lacking. No previous study is known to associate these markers with PD or PRM. Consequently, an explanation for an increment of the M allele in the case group is difficult, since the T allele is the most constantly associated with hypertension. An explanation for this result is offered below.

The AGT gene has been studied in some pregnancy complications, like pre-eclampsia.<sup>22,30,39</sup> In all of these studies, an increment of the T allele was observed. The association of the 235T allele with various diseases and with different

**Table 6**

Haplotype frequencies and linkage disequilibrium in angiotensinogen gene in control and case groups, counts (%).

Haplotype	Observed frequency	Expected frequency	$\chi^2$	p	D'
<b>Controls (n=224)</b>					
174T-235T	221 (49)	239 (53)	29.9	<0.0005	0.8973
174T-235M	178 (40)	160 (36)			
174M-235T	47 (11)	29 (6)			
174M-235M	2 (0)	20 (4)			
<b>Cases (n=89)</b>					
174T-235T	71 (40)	80 (45)	32.7	<0.0005	0.9914
174T-235M	89 (50)	80 (45)			
174M-235T	18 (10)	9 (5)			
174M-235M	0 (0)	9 (5)			

**Key:** Alleles as in table 1. T = threonine; M = methionine; D' = normalised linkage disequilibrium.

AGT circulating levels suggests that the mutation associates with protein function. There are, however, some inconsistencies in this hypothesis, especially regarding investigations in hypertension. Both the 235T and<sup>24,36,37</sup> the 235M allele<sup>28</sup> of the M235T have been associated with hypertension. These observations, along with the present results, which showed an increase of 235M allele in cases (50% *vs.* 40% in controls,  $p=0.027$ ), lead us to consider that the mutations or polymorphisms studied up to now do not necessarily result in altered protein function directly, but they are only linked markers by LD to a still unknown mutation at the AGT gene. The frequency of the 235M allele varies extensively among populations. High frequencies are found in the Middle East and Europe (50%, 70% respectively), low in Africans (2%) and intermediate in Asian (25%) populations. Ethnic genetic diversity probably influences the differential association of this polymorphism and disease. For example, in Europeans and Japanese, the M235T polymorphism is associated with hypertension<sup>65</sup> but not in African Americans.

It has been argued that 235T probably represents the ancient and 235M a newer human allele. The age analysis of these alleles shows that the 235M is recent (22,500–44,500 years).<sup>66</sup> In Native American populations such as Quechuas and Mayan, the frequency of the 235M allele is 20–24%.<sup>67</sup> In this study, the 235M frequency is 40%, probably explained by the genetic admixture in the Mexican Mestizo population. The Mexican population of Jalisco studied here is considered a mixed one, with a 56% Spanish European component.<sup>68</sup> Therefore, population genetic diversity could affect this gene association with diseases. Population diversity should thus be considered in studying RAS-disease association. From the present data, it seems that AGT T174M or REN polymorphisms are not associated with PD-PRM.

In a previous study<sup>18</sup> an association between PD and the ACE I/D polymorphism was suggested ( $p=0.047$ ). This association seems more pronounced in the subgroup of women with PD and PRM ( $p<0.01$  for the distribution of phenotypes in intragroup analysis), although this observation was not corroborated in the present report. It is possible that sample size, small intergroup differences, variable intragroup prevalence of vaginal-caesarean deliveries, as well as random variations, could have affected our ability to corroborate such an association. Since the statistical power of the study is low, it is not possible to ensure that the populations studied are equal in the frequency of the ACE I/D polymorphism. Notwithstanding, considering the intragroup analysis, women with vaginal labour (as opposed to those with caesarean section), showed an increased ACE D allele ( $p<0.01$ ). This difference is probably due to the more homogeneous and representative PD-PRM nature of the vaginal delivery subgroup. Again, a larger sample size is needed to refute or confirm a relationship between ACE and PD-PRM.

Though the AT<sub>1</sub>-receptor A1166C polymorphism did not show a significant intergroup difference, in the intragroup analysis (women with presence *vs.* absence of hypertension/pre-eclampsia/eclampsia) an increase of the C allele was observed in the group with these disorders. Previous studies support this association.<sup>24,52,53</sup> The AT<sub>1</sub>-receptor is found in resistance vessels of the term placenta and it is believed to play a role in the regulation of the fetoplacental circulation. It is therefore thought to have an effect in the placental perfusion. Both AT<sub>1</sub>-receptor and Ang II are associated with vasoconstriction, so AT<sub>1</sub>-receptor might be involved in hypertensive disorders. The reduction of AT<sub>1</sub>-receptor activity diminishes the trophoblastic response to Ang II and prostaglandin secretion, and by means of an inadequate vasodilatation, could induce placental ischaemia and pre-eclampsia.<sup>64</sup>

### Two-locus haplotype analysis

Frequencies of allele combinations at different genomic regions discordant to those determined by random may reflect an additive effect or an interaction between the genes in question. This potential epistatic interaction has been suggested between ACE D/D and AT<sub>1</sub>-receptor A/C, C/C in some diseases.<sup>47,54</sup> This situation may be explained by interactions among genes that are components of complex homeostatic systems like the RAS.<sup>65</sup> The AGT, REN and AT<sub>1</sub>-receptor *loci* are related in their metabolic route. The present results showed that two-locus haplotype proportions are found in agreement with the Mendelian law of independent segregation for unlinked genes, and do not point to a possible genetic interaction among these three genes. The REN-AGT genes in the group of cases showed a moderate LD value (32%,  $p=0.025$ ). This value could be artifactual, since one of its components showed an intergroup distribution difference. The marginal significance ( $p=0.047$ ) in the intergroup comparison of REN-AGT haplotype distribution can be similarly explained by the intergroup difference in AGT polymorphisms (table 6).

### Haplotype analysis within the AGT gene

Haplotype analysis constitutes a powerful tool to identify candidate genetic risk factors for diseases. Although few studies based on the physical map exist, a narrow LD is expected between the polymorphisms of AGT T174M and M235T. In this report, the LD analysis gave similar values ( $p>0.9$ ) in cases and in controls (0.99 and 0.89, respectively). These results are similar to the observed by other authors.<sup>30</sup> With regard to the AGT locus, 174M–235T haplotype has been associated with pre-eclampsia and coronary stent restenosis.<sup>30,69</sup> In the present study, no significant allele frequency difference between cases and controls was found. On the contrary, the 174T–235M haplotype was found in greater proportion in patients with PD-PRM than in controls (50% vs. 40%  $p=0.02$ ). This suggests a PD-PRM-sensitive linked genomic region involving the surroundings of 174–235 nucleotide positions, rather than solely confined to the second one. In the Mexican population studied, such a region would be marked by the 174T–235M haplotype.

### Conclusions

Our results expand the knowledge of genes involved in RAS. Results also indicate that polymorphisms segregate independently, and suggest an association between PD-PRM and the AGT genomic region.

### Acknowledgements

We are very grateful to our laboratory colleagues (Polymorphisms laboratory, Western Biomedical Research Center) for their valuable help in procurement of samples. This study was

supported in part by a special fund from IMSS (FOFOI project number 0038/701), as well as by a CONACYT scholarship to Dr Valdez during her graduate studies.

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## Erratum

The following error appeared in the September 2007 issue of *JRAAS*.

Reference: Roberto Fogari, Amedeo Mugellini, Giuseppe Derosa on behalf of the CANDIA (CANdesartan and DIuretic *vs.* Amlodipine in hypertensive patients) Study Group. Efficacy and tolerability of candesartan cilexetil/hydrochlorothiazide and amlodipine in patients with poorly controlled mild-to-moderate essential hypertension. *JRAAS* 2007;**8**:139-44.

**Author title should state Roberto Fogari, Amedeo Mugellini, Giuseppe Derosa on behalf of the CANDIA (CANdesartan and DIuretic *vs.* Amlodipine in hypertensive patients) Study Group.**

**Throughout the article amlodipine should show 12 (11.8%) and not 13 (12.7%).**