



CLINICAL RESEARCH

## The ACE I/D polymorphism is associated with nitric oxide metabolite and blood pressure levels in healthy Mexican men



Nydia Avila-Vanzzini<sup>a,\*</sup>, Carlos Posadas-Romero<sup>b</sup>,  
Maria del Carmen Gonzalez-Salazar<sup>b</sup>, Carolina Maass-Iturbide<sup>b</sup>,  
Gabriela Melendez-Ramirez<sup>c</sup>, Oscar Perez-Mendez<sup>d</sup>,  
Leonardo Del Valle-Mondragon<sup>e</sup>, Felipe Masso-Rojas<sup>f</sup>, Elvira Varela Lopez<sup>f</sup>,  
Hector Herrera-Bello<sup>g</sup>, Ramon Villavicencio Fernandez<sup>h</sup>, David Cruz-Robles<sup>d</sup>

<sup>a</sup> Department of Echocardiography, National Cardiology Institute "Ignacio Chávez", Mexico

<sup>b</sup> Department of Endocrinology, National Cardiology Institute "Ignacio Chávez", Mexico

<sup>c</sup> Department of Magnetic Resonance, National Cardiology Institute "Ignacio Chávez", Mexico

<sup>d</sup> Department of Molecular Biology, National Cardiology Institute "Ignacio Chávez", Mexico

<sup>e</sup> Department of Pharmacology, National Cardiology Institute "Ignacio Chávez", Mexico

<sup>f</sup> Department of Physiology, National Cardiology Institute "Ignacio Chávez", Mexico

<sup>g</sup> Médica Sur Clinical Foundation, Mexico City, Mexico

<sup>h</sup> Hemodynamic Department, National Cardiology Institute, "Ignacio Chávez", Mexico

Received 26 February 2013; accepted 12 December 2014

### KEYWORDS

ACE I/D  
polymorphism;  
Nitric oxide;  
Healthy subjects

**Abstract** The I/D insertion/deletion polymorphism of the angiotensin-converting enzyme has been related to hypertension. This polymorphism also seems to have gender related implications. Angiotensin II contributes to the production and release of oxygen reactive species that react with nitric oxide, inactivating its effects.

**Objective:** To establish whether the ACE I/D polymorphism correlates with nitric oxide plasma metabolites in healthy men and women.

**Methods:** Among 896 subjects between 18 and 30 years of age range, 138 fulfilled inclusion criteria. The polymorphism was identified by polymerase chain reaction, and blood nitric oxide metabolites were analyzed following the method described by Bryan.

**Results:** Both systolic and diastolic arterial pressures were higher in men than in women (107/67 vs. 101/65 mmHg,  $p < 0.001$ ). In terms of the ACE gene, there were differences in the concentration of nitric oxide metabolites in men with the I/D and D/D genotypes when compared to carriers of the I/I genotype (33.55 and 29.23 vs. 53.74 pmol/ml;  $p < 0.05$ ), while there were

\* Corresponding author at: Department of Echocardiography, Instituto Nacional de Cardiología "Ignacio Chávez", Juan Badiano No. 1, Colonia Sección XVI, Delegación Tlalpan, C.P. 14080, México, D.F., Mexico. Tel.: +52 55 55 73 29 11x1212; fax: +52 55 55730994.

E-mail address: [vazzny74@yahoo.com](mailto:vazzny74@yahoo.com) (N. Avila-Vanzzini).

<sup>1</sup> Contributions by Nydia Avila-Vanzzini and David Cruz Robles to this study are the same and the authors' order is arbitrary.

no significant differences in women when compared by genotype. Men with the *D/D* genotype had higher systolic blood pressure than *I/D* carriers (111 vs. 104 mmHg,  $p < 0.05$ ). We observed no arterial blood pressure differences in women when grouped by ACE genotype.

**Conclusions:** The ACE *D/D* genotype was associated with nitric oxide metabolite levels and systolic blood pressure in clinically healthy men while it had no effect in women.

© 2013 Instituto Nacional de Cardiología Ignacio Chávez. Published by Masson Doyma México S.A. All rights reserved.

## PALABRAS CLAVE

Polimorfismo I/D ECA;  
Oxido Nítrico;  
Población sana

## El polimorfismo I/D de la ECA se asocia con los niveles de metabolitos óxido nítrico y de presión arterial en hombres mexicanos sanos

**Resumen** El polimorfismo inserción/delección del gen de la enzima convertidora de la angiotensina (polimorfismo I/D de la ECA), se relaciona con hipertensión y sus efectos podrían estar asociados al género. La angiotensina II contribuye a la producción y liberación de especies reactivas de oxígeno, que reaccionan con el óxido nítrico (ON), inactivándolo.

**Objetivo:** Conocer si existen diferencias en la concentración de metabolitos de ON en hombres y mujeres sanos que puedan estar influidas por el polimorfismo I/D de la ECA.

**Métodos:** De 896 sujetos de entre 18 y 30 años, 138 cumplieron los criterios de inclusión. El polimorfismo fue identificado usando reacción en cadena de la polimerasa y los metabolitos de ON fueron analizados en sangre usando el método de Bryan.

**Resultados:** Las presiones sistólica y diastólica fueron más elevadas en hombres que en mujeres (107/67 vs. 101/65 mmHg  $p < 0.001$ ). En relación con el genotipo, existieron diferencias significativas en la concentración de metabolitos de ON en los hombres con genotipos I/D, D/D comparados con los portadores del genotipo I/I (33.55 y 29.23 vs. 53.74 pmol/ml, respectivamente;  $p = < 0.05$ ). No hubo diferencias significativas en las mujeres portadoras de los diferentes genotipos. Respecto a la presión arterial, los hombres con genotipo D/D presentaron mayor presión arterial sistólica que aquellos portadores de I/D (111 vs. 104 mmHg,  $p < 0.05$ ). En las mujeres no se observaron diferencias significativas comparándolas por genotipo.

**Conclusiones:** El genotipo D/D de la ECA está asociado con el nivel de metabolitos de ON en plasma y la presión arterial sistólica en hombres clínicamente sanos; esta asociación no se observa en las mujeres.

© 2013 Instituto Nacional de Cardiología Ignacio Chávez. Publicado por Masson Doyma México S.A. Todos los derechos reservados.

## Background

Angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I to angiotensin II (AII), a potent vasoactive peptide with several acute and chronic effects on the cardiovascular system. In humans, the ACE gene is located on chromosome 17q23.3 (dbSNP rs1799752) and exhibits an insertion/deletion (*I/D*) polymorphism consisting of the presence or absence of 287 base pairs in intron 16. This polymorphism has been suggested to modify ACE levels and activity in plasma.<sup>1</sup> Deletion of both alleles (*D/D* genotype) is associated with increased enzymatic activity when compared to the insertion/insertion (*I/I*) genotype. Consequently, heterozygote insertion/deletion (*I/D*) carriers exhibit intermediate ACE concentration and activity.<sup>2</sup>

All has been reported to be involved in the atherosclerotic process, particularly in association with hypertension.<sup>3-6</sup> Several studies have also reported evidence of a significant relation between the ACE *I/D* polymorphism and hypertension in male but not in female subjects.<sup>7-9</sup>

However, this relation has not been consistently observed in males, suggesting that other factors such as age are involved in the impact of the ACE *I/D* polymorphism on blood pressure.<sup>10</sup>

Nitric oxide (NO), synthesized by the endothelium, is important for vascular tone regulation and blood pressure control in humans.<sup>11</sup> All contributes to the production of reactive oxygen species (ROS) that react with NO forming peroxynitrites, thus inactivating the molecule's effects and facilitating the propagation of the oxidative process by free radicals.<sup>12,13</sup>

It is well known that total NO production in patients with essential hypertension is decreased,<sup>14</sup> but little is known on the possible influence of the ACE *I/D* polymorphism on NO production in healthy individuals.

Therefore, we conducted this study to establish whether the ACE *I/D* polymorphism contributes to NO plasma concentrations in healthy men and women. We also searched for differences in blood pressure that have been associated to these genotypes.

## Methods

We selected a total of 896 clinically healthy individuals whose age ranged between 18 and 30, from the blood donor pool of the "Ignacio Chávez" National Cardiology Institute. After preliminary screening, we selected a subgroup that met the following criteria:

- (a) Inclusion criteria: both genders, with no infectious disease manifestations<sup>15</sup> or drug intake within the previous 14 days, no chronic-degenerative diseases or other known disease and with no addictions.
- (b) Exclusion criteria: patients with a body mass index (BMI)  $\geq 25$  kg/m<sup>2</sup>, waist circumference (WC)  $\geq 80$  cm in women or  $\geq 90$  cm in men, systemic arterial blood pressure (systolic, SAP/diastolic, DAP)  $\geq 130/80$  mmHg, glucose  $\geq 110$  mg/dL, total cholesterol (TC)  $\geq 200$  mg/dL triglycerides (TG)  $\geq 150$  mg/dL, pregnant, breastfeeding or menstruating women, patients who refused to sign the informed consent form or had developed any disease within 14 days prior to the study.

The protocol was approved by the Ethics Committee of the National Institute of Cardiology. All participants provided a signed informed consent.

## Laboratory assessment

All patients were instructed to avoid strenuous exercise and eat a light dinner the day before blood samples were drawn. These were obtained in EDTA tubes after a 12 h overnight fast, from an antecubital vein after subjects had been seated for 15 min. Samples were centrifuged at 3000 rpm/20 min at 4°C; the plasma was separated and analyzed or frozen at -80°C until analysis. Plasma glucose, total cholesterol (TC), and triglycerides (TG) were determined by commercially available enzymatic methods. The phosphotungstic acid-Mg<sup>2+</sup> precipitation procedure was used to precipitate apoB-containing lipoproteins before quantifying HDL-cholesterol. Quality control of lipid measurements was assessed by standardization with the Center for Disease Control and Prevention (Atlanta, GA). Low-density lipoprotein cholesterol was estimated in samples with triglyceride values below 400 mg/dl. Serum levels of all lipids were determined within 48 h after drawing the blood samples.

## Data collection

We applied a cardiovascular risk factor questionnaire to our subjects, on their personal and familial habits and that included questions on previous and current diseases. The physical examination included: height, weight, and waist circumference (measured according to the 2006 official Mexican standard procedure).<sup>16</sup> Arterial blood pressure was measured three times in both arms, with 3 min intervals and after a 15 min rest; the average reading was used for analysis.

## Genotyping

DNA extraction was performed in lymphocytes obtained from the blood samples of each patient, following conventional techniques.<sup>17</sup> We used Marre's PCR protocol.<sup>18</sup> As primers, G1IS and FYM generate a specific product for the insertion sequence of 376 base pairs; it has been reported that in heterozygote samples there is preferential amplification of the D allele and although Marre's protocol includes gene verification in the same test, we conducted a second PCR to corroborate the D/D genotype in our samples.<sup>19</sup>

## NO quantification

Nitric oxide (NO) metabolite quantification was performed with Bryan method.<sup>20</sup> Total antioxidant capacity (TAC) was quantified with the CUPRAC method described by Upak et al.<sup>21</sup>

## Statistical analysis

Data were expressed as percentages and averages  $\pm$  standard deviation, or medians according to the variable's distribution. Kolmogorov-Smirnov's test was performed to analyze the distribution of continuous variables. In the case of continuous variables with no normal distribution, comparison between groups was made with Kruskal-Wallis' test.

The Hardy-Weinberg equilibrium (HWE) and comparisons between categorical variables were obtained with the Chi<sup>2</sup> test; continuous variables were analyzed by ANOVA and Bonferroni's post hoc test. Tukey's test was used for multiple comparisons. The association of polymorphisms and NO was analyzed by logistic regression following five inheritance models: co-dominant, dominant, recessive, heterozygous and additive advantage. Results were considered statistically significant if  $p < 0.05$ .

## Results

We recruited 896 apparently healthy subjects in the 18-30 age range. One hundred and seventy-two (172) fulfilled inclusion criteria but 34 were excluded due to abnormal laboratory tests, resulting in 138 healthy volunteers included in the study. The group's anthropometric parameters and biochemical results are shown in Table 1. The group was divided and analyzed by gender. Body mass indices were comparable between men and women but men had greater waist circumference values. Systolic and diastolic blood pressures, LDL-cholesterol and triglycerides were higher in men while HDL-cholesterol and total antioxidant power capacity were lower in men than in women. The general familial risk factors are shown in Table 2 but no gender differences were identified. The polymorphism was in Hardy-Weinberg equilibrium ( $\chi^2 = 1.81$ ;  $p = 0.22$ ). Allelic frequency in this sample of healthy individuals was 59% for the I allele and 41% for the D allele. Genotype frequencies were 37.6% ( $n = 52$ ) for I/I, 42.7% for I/D ( $n = 59$ ), and 19.5% ( $n = 27$ ) for D/D. We found no differences between the groups in the analyzed parameters or upon analysis of the I/D polymorphism in five inheritance models (data not shown).

**Table 1** Anthropometric and biochemical characteristics of the population divided by gender.

	All subjects (n=138)	Men (n=81)	Women (n=57)	p
Age (years)	23 ± 3	24 ± 3	23 ± 2	0.143
Weight (kg)	61.46 ± 7.72	65.33 ± 5.88	55.96 ± 6.64	<0.0001
Height (m)	1.68 ± 0.14	1.71 ± 0.11	1.67 ± 0.18	0.083
BMI (kg/m <sup>2</sup> )	22.41 ± 1.85	22.67 ± 1.65	22.04 ± 2.05	0.057
WC (cm)	78.25 ± 7.07	80.25 ± 6.33	75.40 ± 7.13	<0.0001
SAP (mmHg)	105 ± 10	107 ± 9	101 ± 9	0.001
DAP (mmHg)	66 ± 7	67 ± 7	65 ± 6	0.033
HR (bpm)	65 ± 7	64 ± 7	66 ± 7	0.201
TC (mg/dL)	149.03 ± 25.46	150.11 ± 30.27	147.49 ± 16.51	0.515
C-HDL (mg/dL)	48.37 ± 15.68	44.61 ± 13.89	53.71 ± 16.63	0.001
C-LDL (mg/dL)	85.86 ± 25.11	89.30 ± 27.77	80.96 ± 20.00	0.042
TG (mg/dL)	88.28 ± 32.75	96.54 ± 34.02	76.53 ± 25.77	<0.0001
Glucose (mg/dL)	82.35 ± 8.91	83.53 ± 8.92	80.67 ± 8.71	0.062
PAO (mmol/L)	569.54 ± 293.36	512.20 ± 269.74	651.02 ± 308.36	0.007
NO (pmol/mL)	37.39 ± 28.70	39.76 ± 32.08	34.03 ± 22.91	0.223

Data are expressed as median ± standard deviation. P values were estimated using Student's *T*-test. BMI, body mass index m<sup>2</sup>; WC, waist circumference; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; HR: heart rate; TC, total cholesterol; C-HDL, high density cholesterol; C-LDL, low density cholesterol; TG, triglycerides; PAO, antioxidant power; NO, nitric oxide.

**Table 2** Familial risk factors in the study population.

Risk factor	All subjects (n=138)	Men (n=81)	Women (n=57)
<b>Vascular risk</b>			
HTA (%)	49.3	46.9	52.6
PTCA (%)	15.2	13.6	17.5
Stroke (%)	11.6	12.3	10.5
PVD (%)	31.9	34.6	28.1
<b>Metabolic risk</b>			
DLP (%)	37	33.3	42.1
DT2 (%)	59.4	54.3	66.7
OBE (%)	47.8	43.2	54.4

Data expressed in percentages (%). *p* values were estimated using Chi-square and Pearson's test. HTA, systemic arterial hypertension; PTCA, percutaneous transluminal coronary angioplasty; CVA, stroke; PVD, peripheral vascular disease; DLP, dyslipidemia; DT2, diabetes mellitus type 2; OBE, obesity.

**Table 3** shows the relation between the ACE genotypes and the evaluated parameters when analyzed by gender. We showed significant differences in the systolic arterial pressure of men carrying the *I/D* genotype compared with men with the *D/D* genotype (*p*=0.04). NO levels were lower in men with the *I/D* and *D/D* genotype than in men carrying the *I/I* genotype (*p*=0.03 and *p*=0.022 respectively). Women carrying the *D/D* genotype had a significantly different BMI than *I/D* carriers (*p*=0.026). Logistic regression analysis (uni and multivariate) of risk factors and genotypes showed no statistically significant differences.

## Discussion

ACE is a key enzyme in the renin-angiotensin-aldosterone and kallikrein-kinin systems, and plays an important role in

blood pressure regulation and electrolyte balance.<sup>22</sup> ACE is encoded in chromosome 17q23.3 and the *ACE* gene exhibits different polymorphisms. The *ACE I/D* polymorphism is the most studied, and it may explain up to 47% of the total phenotypic variation in ACE serum levels and determine ACE enzymatic activity.<sup>2</sup> In this study, the participants' selection was very careful, excluding those subjects with physical, hemodynamic or biochemical parameters that were outside of the "normal" range, since the main objective was to assess the levels of NO metabolites in terms of the ACE genotype. Therefore, it was necessary to exclude patients with any entity known to be associated with endothelial dysfunction.

It is important to emphasize that NO has an extremely short life in biological systems (less than 1 s in circulating blood)<sup>23</sup> so consequently, its metabolites (nitrite and nitrate) in blood have been widely used as indicators of endothelial NO synthesis activity.<sup>24,25</sup> For this reason, the concentration of NO was estimated using its metabolites, but this approach is nevertheless unable to distinguish between peroxynitrates and NO synthesis.

The frequency of the I and D alleles of the *ACE* gene, in this sample of healthy individuals, was similar to that previously reported in the Mexican mestizo population.<sup>26-28</sup> Although our study only included participants with normal arterial blood pressure (≤130/80 mmHg), there were statistically significant differences between men and women whereby men's arterial pressure readings were higher than women's. In this regard, Fornage et al.<sup>8</sup> showed that in a group of young subjects with a mean age of 14.8 years, males had a significantly higher systolic pressure than females. Coelho et al.<sup>29</sup> reported that in teenage healthy individuals, males had greater ACE activity, regardless of the genotype; this may explain why the men in our study had higher baseline arterial blood pressures.

Pointing to the *ACE* gene as an attractive candidate in the study of human essential hypertension and in the context of

**Table 3** Analysis of clinical parameters in the population divided by gender and genotype.

Parameter	Male population			Female population		
	<i>III</i> (n=29)	<i>I/D</i> (n=33)	<i>D/D</i> (n=19)	<i>III</i> (n=23)	<i>I/D</i> (n=26)	<i>D/D</i> (n=8)
BMI (kg/m <sup>2</sup> )	22.62 ± 1.65	22.70 ± 1.86	22.68 ± 1.33	22.09 ± 1.67	21.50 ± 2.26	23.63 ± 1.59
WC (cm)	79.66 ± 6.34	80.88 ± 6.78	80.05 ± 5.73	75.61 ± 6.25	74.46 ± 8.02	77.88 ± 6.62
SAP (mmHg)	107.72 ± 8.58	104.85 ± 9.52	111.68 ± 11.02 <sup>a</sup>	102.78 ± 10.25	101.04 ± 9.75	99.88 ± 8.20
DAP (mmHg)	68.38 ± 7.86	66.12 ± 6.95	70.63 ± 7.42	67.35 ± 6.83	63.96 ± 6.63	64.13 ± 5.93
HR (bpm)	63.10 ± 8.23	64.94 ± 8.13	65.05 ± 6.77	66.39 ± 7.69	65.19 ± 7.87	67.88 ± 8.96
TC (mg/dL)	153.66 ± 28.10	148.91 ± 28.29	146.79 ± 37.25	143.87 ± 18.29	147.85 ± 14.40	156.75 ± 15.78
C-HDL (mg/dL)	47.84 ± 12.22	43.33 ± 11.01	41.90 ± 19.58	50.86 ± 15.31	55.15 ± 19.88	57.25 ± 3.80
C-LDL (mg/dL)	90.44 ± 25.72	89.32 ± 25.35	87.53 ± 35.35	80.65 ± 21.73	79.88 ± 20.20	85.37 ± 15.24
TG (mg/dL)	91.17 ± 29.68	98.00 ± 33.38	102.21 ± 43.77	75.09 ± 32.95	74.73 ± 20.80	86.50 ± 14.90
Glucose (mg/dL)	81.59 ± 81.59	84.21 ± 10.28	85.32 ± 7.86	81.87 ± 7.60	80.31 ± 10.08	78.38 ± 7.17
PAO (mmol/L)	494.24 ± 287.57	498.05 ± 224.66	564.22 ± 318.25	689.26 ± 356.20	639.92 ± 299.65	577.19 ± 172.48
NO (pmol/mL)	53.74 ± 46.45	33.53 ± 15.87*	29.23 ± 16.34*	29.52 ± 15.95	36.61 ± 29.66	38.61 ± 11.95

BMI, body mass index m<sup>2</sup>; WC, waist circumference; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; HR, heart rate; TC, total cholesterol; C-HDL, high density cholesterol; C-LDL, low density cholesterol; TG, triglycerides; PAO, antioxidant power; NO, nitric oxide.

Data are expressed as median ± standard deviation. *p* values were estimated using ANOVA and Tukey's test for multiple comparisons.

\* Significant differences in *III* carrier *p* < 0.05.

<sup>a</sup> Significant differences in *I/D* carrier *p* < 0.05.

this disease, some studies have documented differences in arterial pressure between genders.<sup>7-9</sup> In the Suita study, the authors showed that men have higher arterial pressure levels than women in association with the D allele.<sup>9</sup>

In our study of healthy volunteers, we observed that men with the *III* genotype have the highest concentrations of NO metabolites when compared to women with the same genotype and even with men or women with the *I/D* and *D/D* genotype; if the concentration of NO metabolites in reality reflects an increase in NO production, it is possible that the *III* genotype could confer some protection to men carrying the allele and may delay hypertension development. This finding may explain, at least in part, why men with the DD genotype develop systemic arterial hypertension more frequently than men with the *III* genotype as shown in previous studies.<sup>7-9</sup>

With respect to the overall groups' metabolic behavior, men had higher C-LDL and TG levels than women and lower C-HDL and PAO. These findings could be related to gender solely.

### Study limitations

The major pathway for NO metabolism is its stepwise oxidation to nitrite and nitrate. In plasma, NO is almost completely oxidized to nitrite and remains stable for several hours, allowing its measurement. Sample preparation is the most important step in NO metabolite quantification. In this study, extreme care was taken to preserve the samples as described by Bryan, but the possibility of artificially generating NO products or metabolites during sample preparation still exists. This is a limitation in all of the studies that measure NO metabolites, regardless of the method employed. The statistical power of our study was 0.24 because of the decrease in the sample size and this could be another study limitation.

### Conclusions

The *ACE D/D* genotype was associated with NO metabolites' levels and systolic blood pressure in clinically healthy men. High levels of NO metabolites in men with the *III* allele suggest that perhaps, this genotype may delay the manifestation of arterial hypertension in males. On the other hand, these genotypes had no effect in women. As referred in some studies, we conclude that ACE polymorphism has gender implications.

### Funding

No endorsement of any kind received to conduct this study/article.

### Conflict of interest

The authors declare no conflict of interest.

### Privacy policy

The authors declare that they have followed the protocols of the workplace on the publication of patient data.

### References

1. Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, et al. Evidence from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am J Hum Genet.* 1992;51:197-205.
2. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin

- I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest.* 1990;86:1343–6.
3. Straw WB, Dean RH, Ferrario CM. Novel mechanisms linking angiotensin II and early atherogenesis. *JRAAS.* 2000;1: 11–7.
  4. Sekuri C, Cam FS, Ercan E, Tengiz I, Sagcan A, Eser E, et al. Renin–angiotensin system gene polymorphisms and premature coronary heart disease. *J Renin Angiotensin Aldosterone Syst.* 2005;6:38–42.
  5. Butler R, Morris AD, Burchell B, Struthers AD. DD angiotensin-converting enzyme gene polymorphism is associated with endothelial dysfunction in normal humans. *Hypertension.* 1999;33:1164–8.
  6. Todoroki M, Minami J, Ishimitsu T, Ohru M, Matsuoka H. Relation between the angiotensin-converting enzyme insertion/deletion polymorphism and blood pressure in Japanese male subjects. *J Hum Hypertens.* 2003;17:713–8.
  7. O'Donnell CJ, Lindpaintner K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, et al. Evidence for association and genetic linkage of the angiotensin converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham heart study. *Circulation.* 1998;97:1766–72.
  8. Fornage M, Amos CI, Kardia S, Sing CF, Turner ST, Boerwinkle E. Variation in the region of the angiotensin-converting enzyme gene influences interindividual differences in blood pressure levels in young white males. *Circulation.* 1998;97:1773–9.
  9. Higaki J, Baba S, Katsuya T, Sato N, Ishikawa K, Mannami T, et al. Deletion allele of angiotensin converting enzyme gene increases risk of essential hypertension in Japanese men. The Suita Study. *Circulation.* 2000;101:2060–5.
  10. Turner ST, Boerwinkle E, Sing CF. Context-dependent associations of the *ACE I/D* polymorphism with blood pressure. *Hypertension.* 1999;34:773–8.
  11. Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man *Lancet.* 1989;2:997–1000.
  12. Nickening G, The Harrison D. AT1-type angiotensin receptor in oxidative stress and atherogenesis. Part 1. *Circulation.* 2002;105:393–6.
  13. Harrison DG. Endothelial function and oxidant stress. *Clin Cardiol.* 1997;20:11–7.
  14. Forte P, Copland M, Smith LM, Milne E, Sutherland J, Benjamin N. Basal nitric oxide synthesis in essential hypertension. *Lancet.* 1997;22:837–42.
  15. Charakida M, Donald AE, Terese M, Leary S, Halcox JP, Ness A, et al. Endothelial dysfunction in childhood infection. *Circulation.* 2005;111:1660–5.
  16. Secretaría de Salud (México). Norma Oficial Mexicana NOM-043-SSA-2005, servicios básicos de salud. Promoción y educación para la salud en materia alimentaria. Criterios para brindar orientación. Diario Oficial de la Federación, 23 de enero del 2006.
  17. Davis RW, Thomas M, Cameron J, St John TP, Scherer S, Padgett RA. Rapid DNA isolation for enzymatic and hybridization analysis. *Methods Enzymol.* 1980;65:404–11.
  18. Marre M, Jeunemaitre X, Gallois Y, Rodier M, Chatellier G, Sert C, et al. Contribution of genetic polymorphism in the renin–angiotensin system to the development of renal complications in insulin-dependent diabetes: Genetic of the nephropathie Diabetique (GENEDIAB) study group. *J Clin Invest.* 1997;99:1585–95.
  19. Shanmugam V, Sell KW, Saha BK. Mistyping *ACE* heterozygotes. *PCR Methods Appl.* 1993;3:120–1.
  20. Bryan NS, Grisham MB. Methods to detect nitric oxide and its metabolites in biological samples. *Free Radic Biol Med.* 2007;43:645–57.
  21. Apak R, Güçlü K, Ozyürek M, Karademir SE, Altun M. Total antioxidant capacity assay of human serum using copper (II)-neocuproine as chromogenic oxidant: the CUPRAC method. *Free Radic Res.* 2005;39:949–61.
  22. Esther CR, Marino EM, Howard TE, Machaud A, Corvol P, Capecchi M, et al. The critical role of tissue angiotensin-converting enzyme as revealed by gene targeting in mice. *J Clin Invest.* 1997;99:2375–85.
  23. Kelm M. Nitric oxide metabolism and breakdown. *Biochim Biophys Acta.* 1999;1411:273–89.
  24. Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, et al. Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radic Biol Med.* 2003;35:790–6.
  25. Lauer T, Preik M, Rassaf T, Strauer BE, Deussen A, Feelisch M, et al. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. *Proc Natl Acad Sci U S A.* 2001;98:12814–9.
  26. Vargas-Alarcón G, Hernández-Pacheco G, Rodríguez-Pérez JM, Pérez-Hernández N, Pavón Z, Fragoso JM, et al. Angiotensin converting enzyme gene (*ACE*) insertion/deletion polymorphism in Mexican population. *Hum Biol.* 2003;75:889–96.
  27. Martínez-Ríos MA, Peña-Duque MA, Fragoso JM, Delgadillo-Rodríguez H, Pérez-Hernández N, Miranda-Malpica E, et al. No association found between the insertion/deletion of a 287-bp alu repeat sequence within intron 16 of the angiotensin 1 converting enzyme (*ACE*) gene in Mexican patients and binary reestenosis father coronary stenting. *Clin Chim Acta.* 2008;397:65–7.
  28. Avila-Vanzzini N, Espínola-Zavaleta N, Masso-Rojas F, Varela-López E, Casanova-Garcés JM, Kury-Alfaro J, et al. Correlación del polimorfismo (I/D) del gen de la ECA y la función ventricular en pacientes con miocardiopatía dilatada de origen isquémico e idiopático. *Rev Invest Clin.* 2006;58:39–46.
  29. Coelho C, Guerra A, Rego C, Breitenfeld L, Castro E, Rodrigues P, et al. Genetic polymorphism of angiotensin-1 converting enzyme, haptoglobin and angiotensinogen and oxidative stress parameters in 12 to 15 years old adolescents. *Rev Port Cardiol.* 2006;25:677–90.