Research Article

The Relationship Between the Insertion/Deletion Polymorphism of the Angiotensin-Converting Enzyme Gene and Myocardial Infarction in Algerian Population

Ouarda Semmame[1*], Djallila Chellat[1], Hadia Ziada[1], Yasmina Benchabi[2], Dalila Satta[1], Noreddine Abadi[2]
[1]Faculté des sciences de la nature et de la vie, Université des frères Mentouri Constantine, Route Ain el Bey, Constantine, Algérie.
[2]Faculté des Médicine, Université Constantine 3, Laboratoire de Biologie et Génétique Moléculaire, Ain-el-Bey, Constantine, Algérie.
***Corresponding author's E-mail:** s.warda25@yahoo.fr

Accepted on: 27-10-2015; **Finalized on:** 30-11-2015.

ABSTRACT

In the coronary artery diseases (CAD), there are many genes whose products are involved in the development of the disease and for which a genetic polymorphism has been described. Among these, the gene for the angiotensin converting enzyme (ACE) presents a polymorphism of insertion/deletion. The aim of this study was to investigate for the first time a possible association between the ACE genotype and the incidence of myocardial infarction (MI) in Algerian population. In a case-control study, frequencies of the ACE genotypes were compared in 159 unrelated patients with MI and 160 healthy unrelated individuals as a control group. The following variables were determined for each patient: age, sex, BMI, smoking history, diabetes, hypertension, total-cholesterol (Chol), low-density lipoproteins (LDL), high-density lipoproteins (HDL) and triglycerides (TG). The ACE I/D polymorphism was assessed by the PCR amplification. Univariate (chi square) analyses were applied to determine the association between I/D polymorphism (with genotype ID as reference) and MI. P< 0.05 was considered statistically significant. In the present study, the distribution of the DD, ID and II genotypes were 38.77%, 46.25% and 14.96% respectively in patients and 30%, 61.42% and 8.17% respectively in controls. In the overall study sample, the frequencies of DD and ID genotypes were higher than the frequency of II genotype. The DD genotype was significantly more frequent in groups with MI compared to controls (38.99% vs. 30%; p=0.02). A significant association was noted between ACE polymorphism and MI, thus the genotype DD appears to be important cardiovascular risk factor in the Algerian population.

Keywords: angiotensin converting enzyme, ACE I/D polymorphism, Myocardial infarction, risk factors.

INTRODUCTION

Coronary artery diseases (CAD), the main cause of death in industrialised countries, are influenced by environmental and genetic factors. Although the recognition of a number of environmental risk factors has led to important advances in the prevention and treatment of the disease, the role of non-conventional risk factors remains undefined[1,2]. In the last few years, great interest has been focused on genetic factors with the intention of finding common markers that could identify a subgroup of patients at higher risk of death or with a worse prognosis in which new therapeutic timings and interventions could be tested. One important novel genetic factor is the insertion/deletion (I/D) polymorphism of the Angiotensin converting enzyme (ACE)[3].

ACE catalyses the conversion of angiotensin I to angiotensin II, a vasoactive peptide, and inactivates bradykinin, a potent vasodilator[4,5]. The great variety of cardiovascular effects mediated by these vasoactive peptides and the efficacy of ACE inhibitors in the treatment of hypertension and heart failure emphasize the prominent role of ACE in the cardiovascular system[1]. The I/D polymorphism of the ACE gene is characterised by the presence (I) or absence (D) within intron 16 of a 287 bp nonsense DNA domain. This polymorphism results in three genotypes: insertion homozygote (I/I), insertion/deletion heterozygote (I/D) and deletion homozygote (D/D)[6,7].

The ACE I/D polymorphism is not only effective in playing a role in hypertension[8,9] and diabetes[10] but also participates in cardiac complications[11,12] and several pathophysiological conditions[13,14]. Several studies have shown that the DD genotype is associated with higher risk for myocardial infarction (MI)[15-17]. However reports on the association of the ACE gene I/D polymorphism, in addition to being controversial in various other studies[18,19], are relatively lacking among Algerian patients with MI. Therefore, this work was planned to investigate a possible association between the genetic I/D polymorphism of the ACE gene and MI among cases from east region of Algeria.

MATERIALS AND METHODS

Study population

The study population consisted of 319 cases sub-divided into two groups. On the one hand a case group of 159 patients (110 men and 49 women) with a myocardial infarction, diagnosed by experienced cardiologists, taken from those admitted in the cardiology department, the University Hospital of Constantine, Algeria. On the other hand, a control group consisted of 160 healthy unrelated volunteers with no personal or family history of cardiovascular diseases.

Clinical data involved analysis of standard coronary risk factors such as age, sex, diabetes, hyperlipidaemia [total

cholesterol (TC)>2.10 g/l, triglyceride (TG)> 1.50 g/l, Low density lipoprotein (LDL) > 1.30 g/l and High density lipoprotein (HDL) < 0.38 g/l, references of ABADI[20]], smoking history, arterial hypertension (hypertension was defined as a systolic blood pressure of 160 mmHg higher, a diastolic blood pressure of 95 mmHg or higher, or both) and obesity (body mass index BMI >30 kg/m²) were collected from each patient. All the subjects responded to a questionnaire on their medical history and life style. In addition, an informed consent was obtained from all participants. The study was approved by the local Ethical Committee.

Biochemical analysis

The serum concentrations of TC, TG and HDL were measured by standard methods used in the clinical laboratory of the hospital. LDL levels were calculated by the friedewald formula[21].

DNA analysis

Genomic DNA was extracted using NaCl method from EDTA anticoagulated peripheral blood samples. The ACE I/D polymorphism was genotyped by standard polymerase chain reaction (PCR) using the forward primer 5' -CTG GAG ACC ACT CCC ATC CTT TCT-3' and reverse primer 5 '-GAT GTG GCC ATC ACA TTC GTC AGA T-3 '. PCR amplification used 25 µl reaction volumes, and consisted of 2.5 µl of 10X reaction buffer, 20 p M of each primer, 2 m M of deoxynucleotide triphosphates (d NTPs), 1.5 m M of magnesium chloride (Mg Cl_2), 1 unit of taq DNA polymerase (biomatik) and 0.5 µg genomic DNA. PCR was done with an initial denaturation at 94°C for 1 minute. Then the DNA was amplified for 30 cycles with denaturation at 94°C for 30 seconds, annealing at 65.8°C for 30 seconds and extension at 72°C for one minute followed by final extension at 72°C for 8 minute. PCR products were separated on 2% agarose gel stained with ethidium bromide and visualized under ultra violet (UV) light.

In the case of deletion (D allele) and insertion (I allele), a 190 bp fragment band and a 490 bp fragment were obtained, respectively. Therefore, there were three genotypes after electrophoresis: a 490 bp band (II genotype), a 190 bp band (DD genotype) and both 490 bp and 190 bp bands (ID genotype). In heterozygous samples a third band, assumed to represent a heteroduplex DNA product, was commonly seen.

Because the D allele in heterozygous samples is preferentially amplified, each DD genotype sample was confirmed by an additional PCR amplification with a primer pair specific for the insertion sequence (ACE forward 5 ' -TCG GAC ACC AGC CCG CGC ACC TAC-3' and ACE reverse 5 ' -TCG CCA CCG CTC CCA TGC CCA TAA-3'), with identical PCR conditions except for the annealing temperature of 64.5 °C. The reaction yielded a 335 bp amplicon only in the presence of an I allele and no product when the samples were homozygous for DD. 1.38% of ID genotypes were mis identified as DD in the current study.

Statistical analysis

Statistical analysis was performed using the epi info 6.0.

The frequencies of the studied alleles and genotypes among the case patients and controls were counted and were compared by the chi-square test.

Associations were expressed as adjusted odds ratio (OR) with 95% confidence interval (95%).

A p value of <0.05 was considered statistically significant. Numerical values are presented as means +/- standard deviation (SD).

RESULTS

Patients' characteristics

The base line characteristics of both groups are presented in table 1

Table1: Clinical and biochemical characteristics of the study groups

Characteristics	Cases (n=159)	Controls (n= 160)
Age (yr± SD)	57.26 ± 13.66	42.61 ± 15.36
sexe (M/F) (%)	69.18/30.81	58.12/ 41.88
Smoking (%)	35.85	0
Hypertension (%)	24.53	0
Diabetes (%)	27.04	0
Obesity (%)	16.35	12.74
TC(g/l± SD)	1.80 ± 0.43	1.76 ± 0.34 g/l
TG (g/l ± SD)	1.50 ± 0.59	1.12 ± 0.55**
LDL (g/l ± SD)	1.16 ± 0.67	1.01 ± 0.22*
HDl (g/l ± SD)	0.39 ± 0.12	0.44 ± 0.19*

*p< 0.05; ** p<0.01; yr: years; ± SD: standard deviation; M: male, F: female; TC: total cholesterol; TG: triglyceride; LDL: low density lipoprotein; HDL: High density lipoprotein.

The mean age of patients and controls in the study sample were 57.26 ± 13.66 years and 42.61 ± 15.36 years respectively with an age range of 20 and 93 years for cases and 21 and 82 years for controls.

In patients, there were 69.18% males and 30.81 % female. Of them 35.85% were smokers, 27.04% were diabetics, 24.53% were hypertensive and 16.35% obese. Results of the distribution of other several risk factors among patients reflect the expected differences in the prevalence of recognised risk factors.

Cases were older, more likely to be male. Furthermore, their lipid profile was unfavourable compared to controls.

They had a higher TC, TG and LDL levels. Their HDL level was lower than that of controls.

ACE genotyping

Allele and genotype frequencies for the I/D polymorphism of the ACE in a sample of patients with MI compared to controls are shown in table 2.

Results showed that the frequency of D allele carriers (subjects with ID and DD genotypes) was higher than the frequency of the II genotypes in cases and controls. The overall frequencies of genotypes DD, DI and II were 38.99%, 46.54% and 14.46% respectively in patients and 30%, 61.87% and 8.12% respectively in controls.

The observed genotype frequencies are in agreement with frequencies predicted by Hardy-Weinberg equilibrium. Compared to controls, cases had a significantly lower frequency of the ID genotype (46.54 % vs. 61.87 %), but with a significantly higher frequency of the DD genotype (38.99 % vs. 30 %. p= 0.02). In short, it was found that the DD genotype was associated with a significant increase in the risk of MI [OR= 1.73, 95%CI (1.07-2.8)].

Regarding allele frequency, the D allele was the predominant in both group rather than the I allele (62.26% vs. 37.73% respectively in case group and 60.93 % vs. 39.06 % respectively in control group). However when compared to controls, the D allele frequency showed no significant difference between cases and controls.

Comparing ACE I/D genotype frequencies in cases-sub groups related to each single risk factor, such as age, sex, hyperlipidaemia, diabetes, hypertension and obesity are shown in table 3. None of the recognised risk factors shown in table 3 had a significant association with the ACE I/D polymorphism genotype except for smoking.

Table 2: Distribution of ACE genotype and allele frequencies in the study subjects

	Cases (n %)		Controls (n %)		OR (95% IC)	P*
ID	74	(46.54%)	99	(61.87%)	-	-
DD	62	(38.99%)	48	(30%)	1.73 (1.07-2.8)	0.025*
II	23	(14.46%)	13	(8.12%)	2.37 (1.13-4.99)	0.02*
D allele	198	(62.26%)	195	(60.93%)	1.06 (0.77-1.46)	0.72
I allele	120	(37.73%)	125	(39.06%)	-	-

D: deletion; I: insertion; OR (95%CI): odds ratio (95% confidence interval); p*: values obtained through chi-square analysis of the differential genotypes.

Table 3: Association of ACE genotype with other risk factors

	DD	DI	II	
	(n = 62)	(n = 74)	(n = 23)	p
Age	56.73 ±13.86	56.85 ± 14.32	60.04 ± 10.87	-
Sexe (M/F)	47/15	51/23	12/11	-
Hypertension	(12) 19.35%	(19) 25.67%	(8) 34.78%	**0.092**
Smoking	(25) 40.32%	(24) 32.43%	(8) 34.78%	**0.008***
Diabetes	(18) 29.03%	(18) 24.32%	(7) 30.43%	**0.059**
Obesity	(12) 19.35%	(8) 10.81%	(4) 17.39%	**0.13**
TC > 2.10 g/l	(13) 20.96%	(14) 18.91%	(4) 17.39%	**0.059**
TG> 1.50 g/l	(20) 32.25%	(16) 21.62%	(8) 34.78%	**0.078**
LDL> 1.30 g/l	(14) 22.58%	(15) 20.27%	(5) 21.73%	**0.068**
HDL< 0.38 g/l	(19) 30.64%	(18) 24.32%	(8) 34.78%	**0.084**

M: male; F: female; TC: total cholesterol; TG: triglyceride; HDL: High density lipoprotein; LDL: low density lipoprotein; *p< 0.05.

DISCUSSION

Heritable factors, in combination with a number of recognized environmental risk factors, are important determinants of the pathogenesis and natural history of ischemic heart disease. The notion that the presence of the D allele may identify ACE as one of the genes contributing to an increased risk of ischemic heart disease is both intriguing and provocative. The range of hemodynamic effects of an activated renin-angiotensin system, in the context of a correlation between the ACE genotype and plasma ACE activity, is compatible with the concept of an increased risk of MI conferred by the DD genotype. Our results support the postulated role of the ACE genotype as a marker for MI.

The frequency of the ID genotype in our controls group was two times much as the DD genotype (61.87% and 30% respectively). This figure is in agreement with the previous published data in Poland, Japan and Germany in which ID genotype frequency, among the control group, was one and a half to two times as much as the DD[22,23,11].

Regarding the distribution of the ACE genotypes, this study showed a significant association between the DD genotype and the risk of MI [OR= 1.73, 95% CI (1.07 - 2.8); p= 0.025]. This finding is consistent with the results of some[24-27], but not all previous studies[3,28].

Cambien and co-workers were the first to observe an increased prevalence of the ACE DD genotype in patients with a history of MI[15]. Since, many researches on the relation of the ACE I/D polymorphism to CAD and MI tried to confirm those data. Our study is the first in Algeria which investigate the I/D ACE polymorphism association with MI. Reports on this association in Maghreb are in agreements with our results. Nevertheless, the frequency of DD genotype is higher in Tunisia and Morocco compared to our results. For instance, Bennouar noted that the RR was 19.10 with DD homozygotes patients (67.38%), while the RR with ID heterozygote (29.79%) was 6.91 in the Moroccan population[29]. Obtained results in two Tunisian studies showed a raised relative frequency of DD genotype among patients affected with history of MI (51.4% and 52.78% respectively in the two studies)[17,30].

Significant differences can be noticed between the European populations. The Greek population has the highest D allele frequency (0.616)[5], where the lowest (0.435) is reported in the Polish population[31]. In the rest of the European countries, the D allele frequency varies from 0.49 to 0.53[32-34]. The results of a case-control study in first acute myocardial infarction (AMI) cases matched by age and sex to controls with non-cardiac diseases support an increased risk of AMI in Colombian subjects <60 years with ACE DD genotype[35]. Dhar concluded that both DD [OR=2.16; 95%CI (60.60-67.40)] and ID [OR=1.48.95 % CI (93.28 - 97.72)] genotypes of the ACE gene came out to be predisposing factors for the CAD cases[2]. Metta have examined the association of the ACE gene insertion/deletion (I/D) polymorphism in IHD patients with and without smoking and have suggested that the D allele indeed plays a role in predisposing individuals to IHD (OR 1.69, 95% CI 1.139 to 2.517, p=0.009) as compared to I allele[36].

On the other hand, the first study to report results in disagreement with the data of Cambien and our results; was that of Bohn[37], who found a lower frequency of the ACE DD genotype in 234 survivors of MI.

Moreover, Lindpaintner found no association in patients confirmed coronary artery disease and ACE genotype[38]. Likewise, Buraczyńska failed to confirm the role of ACE genotype as an independent risk factor in CHD[39]. In a case-control study of MI patients and controls from the general population as well as a family study neither association nor linkage of the ACE D allele with MI was detected[40].

The results of a meta-analysis performed by Zhang that included Fifty independent publications, with 10 070 stroke cases and 22 103 controls, identified by searching PubMed and Embase through February 2012, provided evidence of the association between the ACE I/D polymorphism and stroke risk, supporting the hypothesis that the ACE I/D polymorphism may be a low-penetrance susceptibility marker of stroke. Subgroup analyses indicated that this risk was more pronounced among Asians, hospital-based studies, and small vessel disease[41]. More recently, Zhao (2015) by a meta-analysis including eight studies have also concluded that the ACE I/D polymorphism may be a risk factor for MI in the Chinese Han population (II vs. DD: OR = 0.40, 95%CI = 0.31-0.53; II vs. DI: OR = 0.72, 95%CI = 0.57-0.91; the dominant model: OR = 1.74, 95%CI = 1.41-2.16; the recessive model: OR = 0.47, 95%CI = 0.38-0.60)[42].

These conflicting results illustrate the difficulty of studies of association in multifactorial pathologies such as MI. Some of the advanced explanations are differences in the criteria used to select the patients and controls and the possibility of differences in the ethnic and genetic background in the samples examined.

CONCLUSION

From this study, we conclude that the ACE gene I/D polymorphism probably have some association with MI. Particularly if integrated with other environmental and genetic risk factor. The study of new risk markers of CAD (ACE I/D polymorphism) provides a potential tool for determining metabolic and genetic susceptibility of atherosclerosis in Algerian population.

REFERENCES

1. Grote K, Drexler H, Shieffer B, Renin-angiotensin system and atherosclerosis, Nephrol Dial transplant, 19, 2004, 770-773.
2. Dhar S, Ray S, Dutta A, BaniSengupta B, Chakrabarti S, Polymorphism of ACE gene as the genetic predisposition of coronary artery disease in Eastern India, Indian heart journal, 64, 2012, 576-581.
3. Settin A, Rizk E, Amr A, Abd-Al-Samad A, Noaman A, Angiotensin-converting enzyme gene insertion/deletion polymorphism in Egyptian patients with myocardial infarction, Renin Angiotensin Aldosterone Syst, 10, 2009, 96-21.
4. Abdelhedi R, Kharrat N, AbdelmoulaBouayed N, Abid L, Abdelmouleh W, TrabelsiSahnoun I, AlFadhli S, Laroussi L, Rebai A, Lack of Association of NOS3 and ACE Gene Polymorphisms with Coronary Artery Disease in Southern Tunisia, Biochem Genet, 51, 2013, 92–100.
5. SekerliE, Katsanidis D, Papadopoulou V, areti1 M, Norma V, Magdalini G, Angiotensin-I converting enzyme gene and I/D polymorphism distribution in the Greek population and a

comparison with other European populations. Journal of Genetics, 87, 2008, 91-93.

6. Rigat B, Hubert C, Corvol P, Soubrier F, PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1), Nucleic acids res, 20, 1992, 1433.
7. Forgarty DG, Mascwell AP, Doherty CC, ACE gene typing, Lancet, 51, 1994, 343(8901).
8. O'Donnell C, Lindpainter K, Larsen M, Rao VS, Ordovas JM, Schaefer EJ, Myers R H and Levy D, 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, 97, 1998, 1766-1772.
9. Lasocki S, Iglaz M, Seince PF, Barrot SV, Vicaut E, Henrion D, Levy B, Desmont JM, Ivan P, Bénessiano J, Involvement of Renin-angiotensin System in pressure-Flow relationship, Anesthesiology, 96, 2002, 271-5.
10. Bhavani BA, Padma T, Sastry B, Krishna Reddy N, Nausheen K, The insertion I/deletion D polymorphism of angiotensin-converting enzyme (ACE) gene increase the susceptibility to hypertension and/or diabetes, Int J Hum Genet, 5(4), 2005, 247-252.
11. Schunkert H, Polymorphism of the angiotensin-converting enzyme gene and cardiovascular disease, J Mol Med, 75, 1997, 867-875.
12. Schieffer B. ACE Gene Polymorphism and Coronary Artery Disease: A question of persuasion or Statistical Confusion? arterioscler. Thromb.Vasc. Bio, 20, 2000, 281-282.
13. Marre M, Jeunemaitre X, Gallois Y, Rodier M, Chatellier G, Sert C, Dusselier L, Kahal Z, Chaillous L, Halimi S, Muller A, Sackmann H, Bauduceau B, Bled F, Passa P, Alhenc-Gelas F, Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes: Génétique de la NephropathieDiabetique (GENEDIAB) study group, J Clin Invest, 99, 1997, 1585-95.
14. Wendling D, Aubin F, Lack of association between familial Mediterranean fever and angiotensin converting enzyme gene polymorphism, Rheumatism, 73, 2006, 517–526.
15. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas F, Soubrier F, Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction, Nature, 359, 1992, 588-589.
16. Stankovic S, Stankovic A, Asanin M, Jovanovic-Markovic Z, Alavantic D, Majkic-Singh N, angiotensin converting enzyme gene polymorphism and activity in patients with ischemic stroke, The journal of the international federation of clinical chemistry and laboratory medicine (JIFCC), 2010.
17. Mehri S, Boussaada R, Mahjoub S, Guemira F, Vuillaumier-Barrot S, Mechmeche R, Durand G, Ben Arab S, Le polymorphisme du gène de l'enzyme de conversion de l'angiotensine et le risque de l'infarctus du myocarde en Tunisie, Antropo, 10, 2005, 75-81.
18. Shafiee SM, Mohsen F, Saeedeh S, Z and H, Hesabi B, Mohebbid A, Angiotensin converting enzyme DD genotype not associated with increased risk of coronary artery disease in the Iranian population, Pathophysiology, 17, 2010, 163–167.
19. Borah PK, Shankarishan P, Ahmed G, Mahanta J, Polymorphism of angiotensin converting enzyme (insertion/deletion) and endothelial nitric oxide synthase (intron 4ab) genes in a population from northeast India, J. Genet, 90, 2011, e105–e109.
20. Abadi N, Contribution à l'étude des lipides dans la population de référence de la ville de Constantine et les maladies cardio-vasculaires, Faculté des médecines-université Constantine, 1998.
21. Friedewald WT, Levy RI, Fredrickson DS, Estimation of the concentration of low-density lipoprotein cholesterol in plasma without the use of preparative centrifuge, ClinChem, 18, 1972, 499–502.
22. Eider J, Cieszczyk P, Ficeka K, Leonska-Duniec A, Sawczuka M, Maciejewska-Karlowskaa A, Zarebskab A, The association between D allele of the ACE gene and power performance in Polish elite, Science & Sports, 28, 2013, 325-330.
23. Mizuiri S, Hemmi H, Inoue A, Yoshikawa H, Tanegashima M, Fushimi T, Ishigami M, Amagasaki Y, Ohara T, Shimatake H, Angiotensin converting enzyme polymorphism and development of diabetic nephropathy in non-insulin dependent diabetes mellitus, Nephron, 70, 1995, 455-9.
24. Ludwig E, Corneli PS, Anderson JL, Marshall HW, Labouel JM, Ward RH, Angiotensin-converting enzyme gene polymorphism is associated with myocardial infarction but not with development of coronary stenosis, Circulation, 91, 1995, 2120-4. 30
25. Agrawal S, Singh VP, Tewari S, Sinha N, Ramesh V, Agarwal S, Gilmour A, Mastana S, Angiotensin converting enzyme gene polymorphism in coronary artery disease in north India, Indian Heart J, 56, 2004, 44-6. 19
26. Hamon M, Fradin S, Denizet A, Filippi-Codaccioni E, Grollier G, Morello R, Prospective evaluation of the effect of an angiotensin I converting enzyme gene polymorphism on the long term risk of major adverse cardiac events after percutaneous coronary intervention, Heart, 89, 2003, 321-5. 22
27. Yang C, Wang X, Ding H. Is coronary artery disease a multifactorial inherited disorder with a sex-influenced trait? Med Hypotheses, 71, 2008, 449–52. 28
28. Dzimiri N, Basco C, Moorji A, Meyer BF, Angiotensin converting enzyme polymorphism and the risk of coronary heart disease in the Saudi male population, Arch Pathol Lab Med, 124, 2003, 531-4. 20
29. Bennouar N, Allami A, Laraqui A, Azeddoug H, El Kadiri N, Benkouka F, Bendriss A, Ghannam R, Benomar A, Fellat S, Benomar M, Implication du polymorphisme génétique de l'apolipoprotéine E et de l'enzyme de conversion de l'angiotensine dans l'athérosclérose, Annales de Biologie Clinique, (62)3, 2004, 295-304. 17
30. ChalghoumA, Noichri Y, Chkioua L, Gammoudi I, Dandana A, Chahed H, Khelil S, Jeridi G, Baudin B, Ferchichi S, Miled A, Étude du polymorphisme intronique de l'enzyme de conversion de l'angiotensine I chez des coronariens

tunisiens, Annales de Cardiologie et d'Angéiologie, 60, 2011, 135-140. 25

31. Zak I, Niemiec P, Sarecka B, Balcerzyk A, Ciemniewski Z, Rudowska, E, Dylag S Carrier-state of D allele in ACE gene insertion/deletion polymorphism is associated with coronary artery disease, in contrast to the C677→T transition in the MTHFR gene, ActaBiochim Pol, 50, 2003, 527-34. 23
32. TiretL, Blanc H, Ruiavet H, Arveiler J, Arveiler D, Luc G, Jeunemaitre X, Tichet J, Mallet C, Poirier O, Plouin PF, Cambier F, gene polymorphisms of the renin angiotensin system in relation to hypertension and parental history of myocardial infarction and stroke: the PEGASE study, Journal of hypertension, 16, 1998, 37-44.
33. Barbalic M, Pericic M, Skaric-Juric T , ACE alu insertion polymorphism in Croatia and its isolates, Coll. Antropologica, 28, 2004, 603-610.
34. Dursunoglu D, Tanriverdi H, Evengul H, Turgut G, Tugut S, Genç O, Kaftan HA, Kliç M, Angiotensin-converting enzyme polymorphism in Turkish male athletes: relationship to left ventricular mass and function, P Cordial, 12, 2005, 145. 33
35. Bautista LE, Ardila ME, Gamarra G, Vargas CI, Arenas IA, Angiotensin-converting enzyme gene polymorphism and risk of myocardial infarction in Colombia, Med SciMonit, 10, 2004, 473-9. 18
36. Metta S, Uppala S, Basalingappa DR, Badeti SR, Mitta G, Mohanty S, Subhadra P, Hasan Q, Association of Angiotensin converting enzyme gene insertion / deletion polymorphism with risk of ischemic heart disease in a population of smokers in southern India, J ClinDiagn Res, 9(4), 2015, GC01-4
37. Bohn M, Berge KE, Bakken A. Erikssen J, Berg K, insertion/deletion (I/D) polymorphism at the locus for angiotensin I-converting enzyme and myocardial infarction, Clin Genet, 44, 1993, 292-297. 31
38. Lindpaintner K, Pfeffer MA, Kreutz R, Stampfer M, Grodstein F, La motte F, Buring J, Hennekens C, A prospective evaluation of an angiotensin-converting enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med, 332, 1995, 706-712.
39. Buraczyńska M, Pijanowski Z, Spasiewicz D, Renin-angiotensin system gene polymorphisms: assessment of the risk of coronary heart disease, Kardiol Pol, 58, 2003, 5-8. 34
40. Holmer SR, Bickebôller H, Hengstenberg C, Rohlmann F, Engel S, Lôwel H, Mayer B, Erdmann J, Baier C, Klein G, Riegger Gunter A, Schunkert H, angiotensin converting enzyme gene polymorphism and myocardial infarction a large association and linkage study, The international journal of biochemistry and cell biology, 35, 2003, 955-962. 35
41. Zhang Z, Xu G, Liu D, Fan X, Zhu W, Liu X, Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism Contributes to Ischemic Stroke Risk: A Meta-Analysis of 50 Case-Control Studies. PLoS ONE, 7(10), 2012, e46495.
42. Zhao W, Ma ST, Cui LQ, Meta-analysis of angiotensin-converting enzyme insertion/deletion polymorphism and myocardial infarction in Han Chinese, Genet Mol Res, 14(3), 2015, 8068-76.

Source of Support: Nil, **Conflict of Interest:** None.