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D Allele and DD Genotype of I /D Polymorphism in The ACE Gene in Patients with Hypertension, Stroke And Cancer Prostate In Libreville: A Concern Given The High Frequencies of these Signatures in Gabonese Population

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Abstract

Background: During the last two decades, the polymorphism of Angiotensin-Converting Enzyme (ACE) gene has been extensively studied among different human populations. In humans, several studies have shown the relationship between this polymorphism and the risk of many serious diseases with a heavy burden of health in developing countries. After analyzing the polymorphism in the population, the present study was also concerned with the investigation of an eventual association between hypertension, stroke, cancer prostate and I/D polymorphism of the ACE gene.

Materials and Methods: Our study population included 163 Baka (pygmy) and 158 Fang (Bantu) from Gabon to evaluate the polymorphism in the country. Concerning the diseases, we included 105 patients and 120 controls for hypertension, 37 patients stroke matched with 50 controls and 97 patients with prostate cancer were recruited. All participants in the study were genotyped for the ACE I/D polymorphism obtained by polymerase chain reaction amplification on genomic DNA.

Results: Our analysis showed that the ACE D allele DD genotype frequencies were highest of all the data so far in human populations. We obtained a frequency of 0.138 for I allele and 0.862 for D allele among pygmy and the frequencies of 0.313 and 0.687 respectively for the I and D alleles. This difference was significant ($p<0.05$). In patients, we revealed the predominance of D allele and DD genotype for hypertension (0.27 for I allele and 0.73 for D allele), for stroke (0.15 for I allele and 0.85 for D allele) and 83% of individuals with cancer prostate carry the D allele. D allele and DD genotype are associated with risk to hypertension whereas allele I seem protective at the occurrence of stroke ($p<0.05$ between healthy and controls).

Conclusion: We show that the D allele and DD genotype were higher in this population. Also theses two signatures may be associated at genetic risk of hypertension, stroke and prostate cancer in this country deprived of human resources for quality care of many patients.

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Introduction

According WHO, of the 56.9 million deaths worldwide in 2016, more than half (54%) are due to the following 10 causes. Ischemic heart disease and stroke are the leading causes of death worldwide, accounting for a total of 15.2 million deaths in 2016 [1]. They have remained the leading cause of death in the world over the past 15 years. In Gabon, a country in central Africa where infectious diseases (malaria, hiv, Ebola virus, sleeping sickness, tuberculosis) have always been a heavy burden in public health to date, the main causes of death. However, in the 2012, the authors noted an epidemiological transition with increasing morbidities of cardiovascular disease (CVD), hypertension and stroke [2]. The damage caused by CVD has become heavy burdens on the health system of Gabon.

Outside of this African region, numerous case-control studies have shown that certain genetic markers such as the angiotensin converting enzyme (ACE) gene can identify individuals at risk by studying its polymorphism.

The angiotensin converting enzyme (ACE) is an important component of renin-angiotensin system and plays an important role in hypertension and other cardiovascular and cerebrovascular disease. In human, the gene encoding ACE is located on the long arm chromosome 17 and comprises 26 exons and 25 introns [3]. The insertion (I)/deletion (D) polymorphism is characterized by the presence (insertion) or absence (deletion) of a 287 base pair (bp) Alu repeat within intron 16, producing three genotypes: II, DD homozygotes and ID heterozygote [4]. A number of research papers have reported a significant association between ACE I/D polymorphism and a series of diseases including hypertension, CVD, type 2 diabetes, stroke, kidney disease, cancer and obesity [5, 6, 7, 8, 9,10]. However few data is available in sub-Saharan Africa.

Gabon does not have enough specialists to take care of these "new patients". On the 9 health regions, only one has cardiologists and neurologists permanently. It is therefore important to know the role played by genetic markers such as ACE gene to encourage health authorities to implement a policy of preventive medicine consequent and in the training to have a sufficient and appropriate human resource.

For the first time in Central Africa especially Gabonese population, the present study aimed to evaluate ACE polymorphism that may be responsible as a genetic risk marker of hypertension, stroke and cancer prostate. We also analyzed the frequencies of these different genotypes and alleles in main population of this country.

Materials and Methods

Ethical Statement

The present study has been performed with the approval of the National committee of Research (N° 0033/2013), Directorate General of Military Health and the Ministry of Public Health of Gabon.

Subjects

Located in Central Africa, Gabon has a heterogeneous population composed of Bantu (farmers) and 3 Pygmy groups (hunters-gatherers). The population is estimated at 1.8 million of which 98% belongs to the Bantu group. In rural areas, all Bantu live along the roads. In some places, there may be a small number of mixed villages because the majority of pygmies live in remote forest villages with small populations.

The recruitment of healthy volunteers was conducted in the region of Woleu Ntem in North Gabon near the frontier with Cameroon where only the Baka

pygmy group is present outside the Fang, the only ethnic group of North Gabon. A total of 321 blood samples, 163 of Baka pygmy and 158 of Fang group, were carried out for two weeks. In each village, only healthy volunteers of all age took part in the study. During enrollments, several informations were collected from each person: age, sex, membership to Bantu or Pygmy populations. The explanations of the aim of the study were made by an ONG- AGAFI composed of people mastering the languages of the two population groups to ensure their autonomy.

With regard to subjects with hypertension, stroke and prostate cancer, patients have been recruited in different hospitals in the capital, Libreville, where specialists in these pathologies are permanently present. For each disease, only individuals reached and followed in the specialized structure were enrolled. The controls are subjects of both sexes aged over 40 years. The purpose and procedures of the experiment were explained and consent was obtained prior to the beginning of the recruitment. With consent from patients or volunteers, 5 ml of peripheral vein blood was collected in EDTA tube. The specimens were immediately stored at - 80°C for genotyping.

DNA Extraction and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from 200 µl EDTA blood of each participant with DNeasy Blood & Tissue Qiagen Kit (Hilden, Germany) according to the manufacturer's protocol and stored at – 20 °c until analysis. Genotyping was performed on the extracted DNA by polymerase chain reaction (PCR) with specific primers. Genotypes of ACE I/D polymorphism were detected according to the methods of Rigat et al. and Wang et al. [4, 11]. The primers sequences were as follows: 5'-CCCAGGCCGGGGAACTCTGTA-3';5'-AGCTCCAGCCCTTAGCTCACCT-3'. Genomic DNA, dNTPs (Sigma, Germany) and TaqDNA polymerase (Invitrogen, USA) were used for PCR mixture. The PCR steps were as the following: initial denaturation step at 94°C for 5 minutes, follow by 30 cycles of denaturation at 94°C for 60 seconds, annealing at 60°C for 45 seconds, extension at 72°C for 90 seconds and final extension at 72°C for 5 minutes. PCR products were separated by electrophoresis on 1.5% agarose gel mix with EZ Evision™ (DNA Dye, Ambresco) and visualized under ultraviolet light to identify the genotype. Amplified products had two fragments: fragment with length of 490 bp was defined I allele, fragment with length of 190 bp was defined D allele. II genotype had only 1 band of 490 bp, ID (heterozygote genotype) had both 490 bp and 190 bp bands, DD genotype had 1 band of 190 bp.

Statistical Analysis

All calculations were done using Graphpad Prism version 6.0 software. The Fishers test was used to calculate for P-value and the two tailed probability levels for statistical significance with $P<0.05$ being considered significant. Hardy-Weinberg equilibrium was not evaluated because several studies showed that the ACE gene was not Hardy-Weinberg equilibrium [12,13].

Results

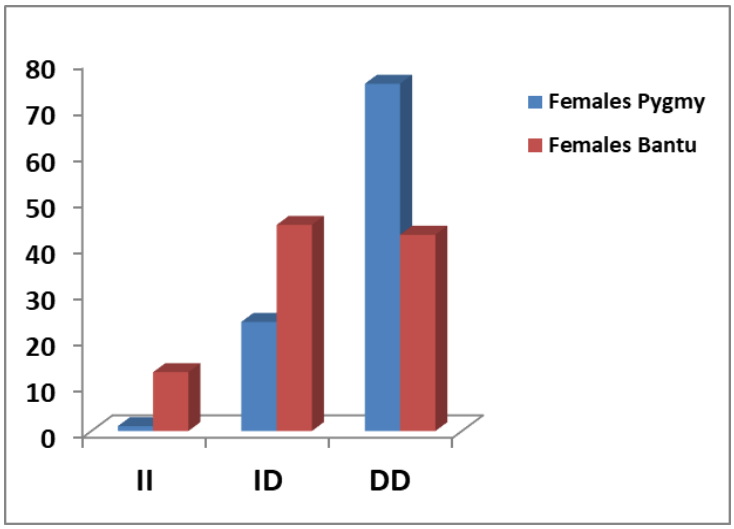
ACE Polymorphisms in Gabonese Population

Several polymorphic genetic systems have been used to study human genetic variation. Here, the frequencies of ACE allele polymorphisms in two groups of population from Gabon were analyzed. Table 1 shows the distribution of genotype and allele frequencies for two ethnic groups of human present in the same environment of Gabon (Central Africa). In two ethnic groups, ACE insertion and deletion alleles were present, we obtained respectively among pygmies [a frequency of 0.138 for I allele and a frequency of 0.862 for D allele. Among Fang population, the frequencies were 0.313 and 0.687 respectively for the I and D alleles. The different frequencies observed in both alleles are very significant between two groups ($P<0.05$). Table 2 shows the different frequencies according to the gender in the two groups of populations. The trends are similar, although a clear difference is observed in frequencies II in females of both groups. The frequency of this genotype in female Fang is 12 times greater than that observed in pygmies. Table 3 shows the frequencies of the insertion deletion alleles in the two Gabonese populations we investigated. We compared them with the allele frequencies from other populations worldwide. The highest D allele frequencies reported so far were for Ivory Coast (0.654) and Kenya (0.618) respectively from Western and East Africa. In European populations (France and England) and Emiratis, the D allele frequencies were moderate (0.592-0.610) and low among Asian and North American populations

Table 1. Genotype and Allele frequencies of the ACE gene in the two groups of Populations from Gabon. ( ): there is either the size of the bands obtained or the number of individuals with the genotype.

Population	n	Genotype II (490bp)	Genotype ID(190-90bp)	Genotype DD (190bp)	Insertion I	Deletion D
Baka pygmy	163	0.013 (2)	0.251(41)	0.736 (120)	0	0.862
Fang	158	0.095 (15)	0.437(69)	0.468 (74)	0	0.687

Table 2. Genotype frequencies of the ACE gene in two groups of populations by sexes



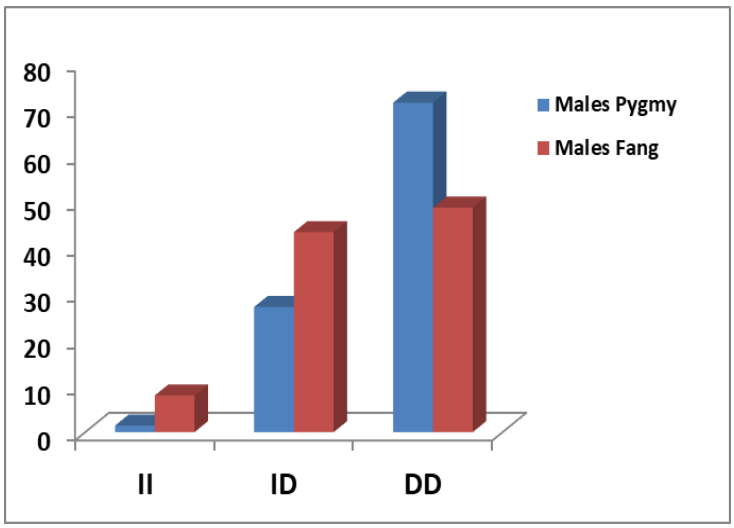


Table 3. Allele frequencies of the ACE gene in the human Populations from different countries.

	Insertion	Deletion	
Population	I	D	Reference
Africa			
Baka pygmy (Gabon)	0.138	0.862	this study
Fang (Gabon)	0.313	0.687	this study
Ivory Coast	0.346	0.654	Santovito et al. (2007)
Kenya	0.382	0.618	Santovito et al. (2007)
African Americans	0.429	0.571	Santovito et al. (2007)
African Brazilians	0.386	0.614	Sakuma et al. (2004)
Europe			
France	0.408	0.592	Santovito et al. (2007)
England	0.41	0.59	Santovito et al. (2007)
Middle East			
Emiratis	0.39	0.61	Bayoumi et al. (2006)
Asia			
China	0.64	0.36	Santovito et al. (2007)
North America			
Mexican Mestizos	0.602	0.398	Santovito et al. (2007)
Australia			
Caucasians	0.455	0.545	Santovito et al. (2007)
Aborigines	0.97	0.03	Santovito et al. (2007)

(0.360-0.398). Very low deletion frequency was reported for the indigenous populations from Australia (0.030). All human populations samples investigated in this study come from Northeast of Gabon in the region of Woleu-Ntem. A comparison among them showed that the gene frequency (0.862) deletion allele from Baka Pygmy was the highest of all the data so far. The deletion frequency obtained in ethnic group Fang (Bantu) from Gabon, although close to those of other African countries is slightly elevated.

ACE Polymorphisms in Patients and Controls

In Table 4, we have the frequencies of genotypes and alleles in patients and controls in hypertensive and stroke subjects. In hypertensive patients, the differences are significant at the level of the two alleles (I and D) and at the level of the two homozygous genotypes (II, DD). 73% of cases with hypertension carry the D allele. We had 37 patients with stroke, 75% of whom had hemorrhagic stroke and 25% ischemic stroke; the youngest patient is a 32 year old man and the oldest is a woman (75 years old). In these patients (85% with D allele), significant differences are observed only in genotype II and the allele I. Regarding subjects with cancer prostate, we were unable to obtain a sufficient number of controls that could be used to make a comparison. However, of the 97 patients recruited (32 to 90 years), we obtained the following frequencies: 0.65, 0.30 and 0.005 respectively for DD, ID and II genotypes. 83% of individuals with cancer prostate carry the D allele.

Discussion

The association between ACE gene polymorphism and several diseases has been noted in several studies around the world. This link has established that some signatures of this polymorphism may be the risk factors. Knowledge of the risk factors of a disease can help to improve the policy of preventive medicine in a country's health system. Also it is known that the polymorphism of this gene varies by region, ethnic group and race [12, 13, 14].

In this study, we analyzed for the first time in Gabon, a country of Central Africa, the possibility of the existence of an association between the polymorphism of the ACE gene and certain diseases such as hypertension, stroke, and prostate cancer that causes huge damage in the Gabonese population. In the two groups comprising the population of this country, we obtained frequencies of 0.313 for the I allele and 0.687 for the D allele in the Bantu (Fang) whereas for the Pygmies these frequencies are respectively 0.138 and 0.862. The difference in the frequencies of these two alleles is highly significant ($p<0.05$). The result of this study showed a high frequency of D allele in Gabonese population. This allele has been reported as associated with cardiovascular diseases and hypertension in North Africa and Asia [15, 16, 17] The pygmies are considered to be largest group of mobile hunter-gatherers of Africa. They dwell in equatorial forests and are characterized by their short mean stature. However, little is known about their cardiovascular disease status because the influence of lifestyle on global burden of cardiovascular diseases has been well established [18, 19].

The results of our study showed a significant association of the gene ACE DD genotype and D allele with hypertension. Also the significant difference observed at genotype II and allele I suggested that these signatures are protective factors to hypertension. Our results therefore confirm that D and DD genotype are risk factors for hypertension as has been observed in several studies in other regions [20, 21, 22].

In stroke cases, we also have a predominance of the D (85%) allele and the DD genotype (70%). however, a significant difference in frequencies is observed only at the level of the I allele and genotype II between the patients and the controls. This result suggests that these genetic signatures are protective factors against stroke in this region. Previous studies in other ethnic populations have a strong association of DD genotype and D allele with stroke [23, 24]. Of the 37 patients, 75% had hemorrhagic stroke and 25% had ischemic stroke, this confirms the observation that hemorrhagic stroke is often suffered by a predominantly DD genotype population [25]. In India, however, it has been shown that ACE II genotype and I allele predicts ischemic stroke among males [26]. Hypertension is one of the leading factors in the onset of stroke (40%) and given our results on hypertension, we can hypothesize that apart from this factor, other factors such as diabetes, smoking, alcoholism, physical inactivity may participate in the epidemiology of stroke in this region especially since according to WHO, Gabon is the first

Table 4. Genotype and allele frequencies of ACE I/D polymorphisms in patients and controls

Hypertension					
ACE	Patients (n=105)	Controls (n= 120)	p-value	Relative Risk (Confidence interval)	OR (Confidence interval)
II	6 (0.06)	38 (0.32)	p<0.0001	0.2493 (0.1171 to 0.5307)	0.1308 (0.05266 to 0.3248)
ID	43 (0.41)	64 (0.53)	0.0636	0.7648 (0.5735 to 1.020)	0.6069 (0.3575 to 1.030)
DD	56 (0.53)	18 (0.15)	p<0.0001	2.332 1.791 to 3.037)	6.476 (3.445 to 12.17)
I	55 (0.27)	140 (0.42)	p<0.0001	0.4640 (0.3633 to 0.5927)	0.2535 (0.1698 to 0.3784)
D	155 (0.73)	100 (0.58)	p<0.0001	2.155 (1.687 to 2.753)	3.945 (2.643 to 5.890)
Stroke					
ACE	Patients (n=37)	Controls (n= 50)	p-value	Relative Risk (Confidence interval)	OR (Confidence interval)
II	0 (0)	9 (0.18)	0.0064	00	0.05825 (0.0032 to 1.036)
ID	11 (0.30)	24 (0.48)	0.0858	0.6286 (0.3591 to 1.100)	0.4583 (0.1868 to 1.124)
DD	26 (0.70)	17 (0.34)	0,0008	2.419 (1.373 to 4.260)	4.588 (1.835 to 11.47)
I	11(0.15)	42 (0.35)	0.004	0.3986 (0.2292 to 0.6932)	0.2411 (0.1135 to 0.5124)
D	63 (0.85)	58 (0.65)	0.106	2.508 (1.443 to 4.362)	4.147 (1.952 to 8.813)

alcohol consuming country in Africa. We can also consider the existence of interactions between the ACE gene and other genes such as the apolipoprotein gene with which a synergistic effect of these polymorphisms and hypertension in the pathogenesis of early ischemic stroke has been determined [27, 28].

In prostate cancer cases, a predominance of allele D (80%) and genotype DD (65%) is also noted. Despite the lack of data on controls, it is clear that this D allele seems to play again an important role in the epidemiology of this disease, especially since several studies have shown that the DD genotype and the D allele are prostate cancer risk factors [29, 30]. Also unlike hypertension and stroke where the gender factor can affect the results from the ACE [31, 32] gene polymorphism, in the case of prostate cancer, an association with D theses signatures of **I/D** polymorphism seems conceivable as has been noticed in other regions.

Conclusion

The DD genotype and the D allele may be associated with a genetic risk of hypertension, stroke and prostate cancer in this population where theses signatures were highest of all the data so far in human populations. However, further studies considering different ethnic groups, environmental factors with large samples are required to confirm this finding.

Author Contributions

B.O, B.BM, LYM and E.N.M designed research; L.Y.M, B.O, TMM, BMO, EL, JEEN, RM, PN, CN, J.M and B.O performed research; B.O, E.N.M, B.BM. Analyzed data; B.O, B.B, J.M, BMO and E.N.M wrote the paper.

Competing Interests

The authors have declared that they have no competing interests.

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References

1. WHO. (2016). Preventing chronic disease: A vital investment. Geneva, Switzerland: World ealth organization. WHOGlobal Report: available at http:// www.who.int/chp/chronic_disease_report/en/.
2. Ngoungou EB, Aboyans V, Kouna P, Makandja R, Ecke Nzengue JE, Ndong Allogo C, Laskar M, Preux PM, Lacroix P. (2012). Prevalence of cardiovascular disease in Gabon: A population study. Archives of Cardiovascular Disease .105: 77-83.
3. Hubert C, Houot AM, Corvol P, Soubrier F. (1991). Structure of the angiotensin I-converting enzyme gene. Two alternate promoters correspond to evolutionary steps of a duplicated gene. J Biol Chem Aug 15, 266(23):15377-83.
4. Rigat B, Hubert C, Corvol P, Soubrier F. (1992). PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res; 20: 1433.
5. Kennon B, Petrie JR, Small M, Connell JM. (1999). Angiotensin-converting enzyme gene and diabetes mellitus. Diabet Med 16(6):448-58.
6. Medeiros R, Vasconcelos A, Costa S, Pinto D, Lobo F, Morais A, Oliveira J and Lopes C. (2002). Linkage of Angiotensin I-Converting enzyme gene insertion/deletion polymorphism to the progression of human prostate cancer. J Pathol 330-335.
7. Inanir A, Basol N, Karakus N, Yigit S. (2013). The importance of association between angiotensin-converting enzyme (ACE) Gene I/D polymorphism and diabetic peripheral neuropathy. Gene 530 (2):253-6.
8. Mengesha HG, Petrucka P, Spence C, Tafesse TB. (2019). Effects of angiotensin converting enzyme gene polymorphism on hypertension in Africa: A meta-analysis and systematic review. PLoS One. 14 (2):e0211054.
9. Sayed-Tabatabae FA, Schut AF, Vasquez AA, Bertoli-Avella AM, Hofman A, Witteman MJ, et al. (2005). Angiotensin converting enzyme gene polymorphism and cardiovascular morbidity and mortality: The Rotterdam Study. J Med Genet, 42:26-30.

10. Cambien, F, Poirier O, L. Lecerf et al. (1992). Deletion polymorphism in the gene angiotensin-conventing enzyme is a potent risk factor for myocardial infarction. Nature 359: 641-644.
11. Wang K, Li Y, Dai C, Wang K, Yu J, Tan Y, Zhang W, Yu XF. (2013). Characterization of the relationship between APOBEC3B deletion and ACE Alu insertion. PLoSOne, 8(5):e64809.
12. Bayoumi RA, Simsek T, M Yahya et al. (2006). Insertion-deletion polymorphism in the angiotensin converting enzyme (ACE) gene among Sudanese, Somalis, Emiratis, and Omanis. Hum Biol: 78 (1):103 -108.
13. Santovito A, Burgarello C, Caravatti GA, Ouattará M, Cervella P, Pisano G, Salvarani B, Delpero M. (2007). ACE and LRPAP1 insertion-deletion polymorphisms in a northern Ivory Coast population. Hum Biol. 79 (6):699-706.
14. Barley J, Blackwood A, Carter ND, Crews DE, Cruickshank JK, Jeffery S, Ogunlesi AO, Sagnella GA. (1994). Angiotensin converting enzyme insertion/deletion polymorphism: association with ethnic origin. J Hypertens. 12 (8):955-7.
15. Mostafa MA, El-Nabiel LM, Fahmy NA, Aref H, Shreef E, Abd El-Tawab F, Abdulghany OM. (2016). ACE Gene in Egyptian Ischemic stroke patient. J Stroke Cerebrovasc Dis. 25 (9):2167-71.
16. Munshi A, Sultana S, Kaul S, Reddy BP, Alladi S, et al. (2008). Angiotensin-converting enzyme insertion/ deletion polymorphism and the risk of ischemic stroke in a South Indian population. J. Neurol. Sci. 272, 132-135.
17. Saidi S, Zammiti W, Slamia LB, Ammou SB, Almawi WY, Mahjoub T. (2009). Interaction of angiotensin-converting enzyme and apolipoprotein E gene polymorphisms in ischemic stroke involving large-vessel disease. J Thromb Thrombolysis. 27 (1):68-74.
18. Daweber TR, Meadors GF, Moore FE JR. (1951). Epidemiological approaches to heart disease: The Framingham Study. Am. J. Health Nations Health, 41:279-281.
19. Kesteloot H, Ndam N, Sasaki s, Kowo M, Seghers V. (1996) A survey of blood pressure distribution in pygmy and Bantu populations in Cameroon. Hypertension, 27:108-113.
20. Zawilla N, Shaker D, Abdelaal A, Aref W. (2014). Angiotensin-converting enzyme gene polymorphisms and hypertension in occupational noise exposure in Egypt. Int J Occup Environ Health. Jul;20(3): 194-206.
21. Yun FZ, Hui Y, Xiao PH, jing LM, Jong Z et al. (2003). Association study of angiotensin converting enzyme gene polymorphism with elderly diabetic hypertension and lipids levels. Lipids in health and Disease 12:187.
22. Bhavani BA, Padma T, Sastry BKS, Reddy NK, Nausheen K. (2005). 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):247–252.
23. Sharma P. (1998). Meta-analysis of the ACE gene in ischaemic stroke. J Neurol Neurosurg Psychiatry. 64 (2):227-30.
24. Catto A, Carter AM, Barrett JH, Stickland M, Bamford J, Davies JA, Grant PJ.(1996). Angiotensin-converting enzyme insertion/deletion polymorphism and cerebrovascular disease. Stroke. 27(3):435-40.
25. Das S, Roy S, Sharma V, Kaul S, Jyothy A, Munshi A. (2015). Association of ACE gene I/D polymorphism and ACE levels with hemorrhagic stroke: comparison with ischemic stroke. Neurol Sci. 63(1) :137-42.
26. Vijayan M, Chinniah R, Ravi PM, Mosses Joseph AK, Vellaiappan NA, Krishnan JI, Karuppiah B. (2014). ACE II genotype and I allele predicts ischemic stroke among male in South India. Meta Gene 2:661-669.
27. Stankovic S, Stankovic A, Asanin M, Jovanovic-Markovic Z, Alavantic D, Majkic-Singh N. (2010). The Interaction of Apolipoprotein E and Angiotensin I-Converting Enzyme Dna Polymorphisms with Hypertension on Early Ischemic Stroke Risk. EJIFCC. 21(1):13-8.
28. Hu DC, Zhao XL, Shao JC, Wang W, Qian J, Chen AH, Zhang HQ, Guo H, Jiang J, Li HY. (2014).

Interaction of six candidate genes in essential hypertension. Genet Mol Res. 20;13(4):8385-95.

29. Hasanzad M, Samzadeh M, Jamaldini SH, Haghdoost AA, Afshari M, Ziaei SA. (2012). Association of angiotensin I converting enzyme polymorphism as genetic risk factor in benign prostatic hyperplasia and prostate cancer. Genet Test Mol Biomarkers. 16(7):770-4.
30. Wang ZY, Li HY, Jiang ZP, Zhou TB. (2018). Relationship between angiotensin-converting enzyme insertion/deletion gene polymorphism and prostate cancer susceptibility. J Cancer Res Ther. 14 (Supplement):S375-S380.
31. Liu J, Lu FH, Wen PE, Jin SK, Wu F, Zhao YX, Liu ZD, Sun SW.(2005). The impact of gender factor on the candidate gene study of essential hypertension. Zhonghua Xin Xue Guan Bing Za Zhi. 33(11):1010-3.
32. Dhanachandra Singh Kh, Jajodia A, Kaur H, Kukreti R, Karthikeyan M. (2014). Gender specific association of RAS gene polymorphism with essential hypertension: a case-control study. Biomed Res Int. : 538053.