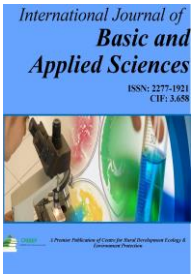
Content is available at: CRDEEP Journals
Journal homepage: http://www.crdeepjournal.org/category/journals/ijbas/

International Journal of Basic and Applied Sciences
(ISSN: 2277-1921) (Scientific Journal Impact Factor: 6.188)

UGC Approved-A Peer Reviewed Quarterly Journal



Full Length Research Paper

An Association Study ACE Gene Polymorphism with Hypertension in Vindhyan Population

Ramji Soni and R K Garg*
Department of Centre for Biotechnology Studies, APS University, Rewa, Madhya Pradesh, India.

ARTICLE DETAILS

Corresponding Author:
R K Garg

Key words:
ACE gene, RAAS, BP, PCR, Hypertension

ABSTRACT

Angiotensinogen (AGT) enzyme comprises a vital module of RAAS system that effectively controls the blood pressure and related cardiovascular functions. Ample association studies have reported the importance of AGT variants in cardiovascular and non-cardiovascular adversities. The Renin-Angiotensin-Aldosterone System (RAAS) is involved in the pathophysiological process of EHT. The RAAS consists of angiotensinogen (AGT), angiotensin I (AngI), angiotensin II (AngII), AngI to AngII converting enzyme (ACE), AngII type 1 receptor (R1 AngII), AngII type 2 receptor (R2 AngII) and renin (REN). RAAS exerts its effects through neurohumoral mechanisms to regulate blood pressure (BP) in the pathophysiological process of cardiac remodeling. The angiotensin converting enzyme (ACE) is a gene situated on chromosome 17q23 that shows a 287-bp repeated Alu sequence insertion (I) or deletion (D) polymorphism in intron 16. The PCR products were analyzed by electrophoresis on 3% agarose gel then visualized under a UV transilluminator with 100-bp ladder. The amplification products were; 490 bp in of the I (insertion) allele and 190 bp of the D (deletion) allele. Genotype frequency between Hypertension patient and healthy control groups were slightly different and but not significantly associated with Hypertension ($\chi 2$=2.025,P=0.3633). Thus allele frequency ($\chi 2$=0.8091, P=0.3684) and carriage rate ($\chi 2$=0.4590, P=0.4981) were also not significantly different between both case and control groups. An odds ratio of 0.7860 in Hypertension group respectively for '*II*' genotype indicated a no role in protective effect of this wild type genotype. In addition, all genotype ID and DD was also not showing protective effects. Data from allele frequency of allele '*I*' was found in large in the population.

1. Introduction

Gene polymorphisms associated with the renin–angiotensin–aldosterone system (RAAS) have been extensively studied in hypertension patients, due to therapeutic potential of targeting the RAAS and slowing down the disease progression. Hypertensive rat had revealed an elevated level of ACE gene expression in the tissues and is substantiated by experimental clinical studies for a positive correlation between ACE I/D polymorphism and hypertension. Aim: To determine whether the polymorphic variant of ACE gene in intron 16 confers susceptibility to essential hypertension **[2].** I/D polymorphism at the

* Author can be contacted at: *Department of Centre for Biotechnology Studies, APS University, Rewa, Madhya Pradesh, India.*

Received: 03-December 2023; Sent for Review on: 06-December 2023; Draft sent to Author for corrections: 24-December 2023; Accepted on: 02-01-2024

Online Available from 04 January 2024

DOI: 10.13140/RG.2.2.26781.28642

locus intron 16 of the ACE gene were amplified from the genomic DNA using polymerase chain reaction and gel electrophoresis methods and were examined in a case–control approach. Suitable descriptive statistics was used for different variables. The roles of genes in the RAAS, including angiotensin-converting enzyme (ACE), angiotensinogen (AGT), angiotensin II receptor type 1 (AGTR1), and aldosterone synthase (CYP11B2) genes in hypertension **[1-3].**

The 4 polymorphisms in the RAAS selected because of their roles in the pathogenesis of hypertension. (e reference SNP identification (rs) of ACE, rs1799752, an insertion/deletion (I/D) polymorphism, has been associated with many diseases. (e D allele, which has increased activity, not related to increased generation of angiotensin II, is associated with increased risk of hypertension and preeclampsia, among others. AGT is converted by ACE to angiotensin II, a potent vasoconstrictor; M235T (rs699) is a nonfunctional polymorphism, but 235 is in linkage equilibrium with −6A. AGT haplotype 1, which contains the variants −217A, −6A, +507G, and +1164A, is associated with increased BP in humans and transgenic mice. Pro-hypertensive effect of angiotensin II occurs by occupation of AGTR1, resulting in vasoconstriction and sodium retention. Polymorphisms of AGTR1 such as rs5186 are associated with hypertension **[4].** Aldosterone synthase, which is needed to synthesize aldosterone, has a genetic polymorphism, CYP11B2 rs1799998, that is associated with hypertension. However, a recent meta-analysis was not able to show the association of the SNPs of ACE, AGT, and CYP11B2 genes and hypertension. AGT is involved in the pathogenesis of EHT, is a growth factor for myocytes and it induces cardiac hypertrophy. The *M235T* genetic variant (chr 1q42-q43) is a point substitution in which methionine is replaced by threonine at position 235. Hypertensive patients carrying the *M235T*-AGT genotype have an increased risk of LVH and IHD, as they exhibit increased plasma AGT levels. The *M235T*-AGT genotype is associated with increased AGT levels, *TT* homozygotes are at a high risk for LVD and IHD. Male and female athletes carrying the *TT*-AGT genotype exhibit LVH as an adaptive response to exercise **[5,7].** ACE plays a role in converting AngI to AngII. ACE and AngII are involved in the pathophysiological process of LVH, independently of hemodynamic factors. Insertion/deletion (*I/D*) at position 16 of the ACE gene (chr 17q23.3) influences the activity of ACE and AngII. ACE accelerates cardiac fibrosis progression and the increase in LV mass, which is why hypertensive homozygous carriers of the *DD* allele have an increased risk for LVH and IHD. This aspect of the increased risk of LVH associated with the *DD* genotype differs depending on the geographical area and race, with Caucasians being at highest risk. *I/D*-ACE polymorphisms are associated with the increase in LV mass in hypertensive and normotensive patients. Normotensive athletes carrying the *DD* genotype have a greater increase in heart muscle mass compared with athletes carrying the *I/I* genotype **[4-8].**

2. Materials and Methods

2.1 Study population:

The study population consisted of 210 unrelated subjects comprising of 90 Hypertension patients and 120 ethnically matched controls of central Indian population were included in this study. In this region Hindu, Muslim and some Sikh peoples are mainly living but most people's belong to Hindu religion in this region.

2.2 Blood collection and plasma/serum separation:

Venous blood samples were obtained from the subjects after 12 hours of overnight fasting in vacutainers with and without appropriate anti-coagulants. Immediately, plasma and serum from the respective vacutainers were separated by centrifuging the tubes at 1000 rpm for 10 min. at 4˚C

2.3 Molecular Laboratory Analysis

2.3.1 Method for DNA isolation

Genomic DNA was extracted from whole blood by the modification of salting out procedure described by Miller and coworkers (Miller *et al*. 1988). Frozen blood sample was thawed at room temperature. 0.5 ml. of whole blood sample was suspended in 1.0 ml. of lysis buffer (0.32 M Sucrose, 1 mM Mgcl2, 12 mM Tris and 1% Triton-X-100) in a 1.5 ml. microcentrifuge tubes. This mixture was mixed gently by inverting the tube upside down for 1 min. The mixture was than allowed to stand for 10 min. at room temperature to ensure proper lysis of cells. The mixture was centrifuged at 11,000 rpm for 5 min. at 4˚C to pellet the nuclei. The supernatant was discarded carefully in a jar containing disinfectant, as pellet formed is loosely adhered to the bottom of centrifuge tube. The pellet was resuspended in 0.2 ml. of lysis buffer and recentrifuge at 11,000 rpm for 5 min. The pellet was than dissolved in 0.2 ml. of deionized autoclaved water and mixed thoroughly on vortexer. The mixture was

centrifuged at 14,000 rpm for 1 min. at 4˚C. Supernatant was discarded to gain an intact pellet. To the above pellet, 80 µl. of proteinase K buffer (0.375 M Nacl, 0.12 M EDTA, pH 8.0) and 10 µl. of 10% SDS (10% w/v SDS, pH 7.2) was added. Mixture was well frothed with the help of micro tip to allow proper lysis of pelleted nuclei. After digestion was complete, 100 µl. of saturated cold 5M Nacl was added and shaken vigorously for 15 sec. To the above mixture 0.2 ml. of deionized, autoclaved water and 0.4 ml. of phenol-chloroform (4:1 v/v) was added to remove most of the non nucleic acid organic molecules. Microcentrifuge tube was inverted upside down until the solution turned milky. Phases were separated by centrifuging the above mixture at 12,000 rpm for 10 min. at 4˚C. Aqueous (top) layer was saved and transferred in another microcentrifuge tube. Transferring of any interface layer was avoided. To the aqueous layer, 1 ml. chilled absolute ethanol was added and the tube was inverted several times until the DNA precipitated. DNA precipitates like thread. This was centrifuged at 14,000 rpm for 4 min. at 4˚C to pellet the DNA thread. Supernatant was discarded. The pellet was washed twice with 1 ml. of 70% alcohol. The mixture was again centrifuged at 14,000 rpm for 1 min. 4˚C. Supernatant was discarded and pellet was air dried for 10-20 min. The pelleted DNA was rehydrated in 100-200 µl. of TE buffer pH 7.4 (10 mM Tris-HCL pH 7.4, 1mM EDTA, pH 8.0). DNA was allowed to dissolve overnight at 37˚C before quantization.

2.3.2 Determination of quality and quantity of isolated DNA:

The isolated DNA is to be used for PCR based study. Therefore its suitability for PCR along with its size heterogeneity is among the most important criterion for purity. As a matter of general practice all DNA preparations were tested for quality and quantity measures, as described in the following paragraphs.

2.3.3 Quantitation by UV spectrophotometry:

The isolated genomic DNAs were then tested for purity by measuring their absorbance values at 230 nm, 260 nm, 280 nm and 300 nm using a UV visible spectrophotometer (Systronic, India). A DNA preparation was considered to be good if it had A 260 nm / A 280 nm ratio as approximately 1.8 and A 300 nm was 0.1 or lesser. The absorbance at 260 nm was used to calculate the amount of DNA, using the relationship that double stranded DNA at 50µg/ml concentration has an absorbance= 1.0 at 260 nm.

2.3.4 Agarose Gel Electrophoresis:

Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appears as single band. A horizontal agarose slab gel electrophoresis apparatus (Bangalore Genei, Bangaore, India) was used. In brief, 4-5 µl of each genomic DNA was loaded on 0.8 agarose (0.8 % w/v, Sigma) containing ethidium bromide solution (0.5 µg/ml) and electrophoresis was done at 80 V in 1x TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA). Lambda DNA *Eco*RI / *Hind* III double digest (Bangalore Genei, Bangalore, India) was used as molecular weight marker after completion of electrophoresis, the DNA bands were visualized and photographed using an UV transilluminator (312 nm) and gel documentation system (Vilber Lourmate, Cedex 1, France) respectively.

2.3.5 Polymorphism screening:

In general, the genomic DNA extracted from peripheral blood of healthy individuals and diseased individuals was subjected to PCR followed by restriction digestion and electrophoresis to genotype both the groups for relevant gene of interest. All the PCRs were carried out in a PTC 200 thermal cycler (MJ Research Inc. USA). PCR is a rapid, inexpensive and simple mean of producing relatively large copy number of DNA molecules from the small amounts of source DNA material, even when the source DNA is of relatively poor quality. Due to the extreme sensitivity, precautions were taken against contamination of the reaction mixture with the trace amounts of DNA, which could serve as an unwanted template. Appropriate negative control was included in each PCR run carried out for each gene, to monitor this contamination of PCR mix to avoid any false positive results. The negative control used for PCR contained whole PCR reaction mix except target DNA which was replaced by HPLC purified water free of RNase, DNase, and any contamination from any other source resembling the gene sequence.

Subsequently restriction enzyme digestion was performed by incubating the double stranded DNA with appropriate amount of restriction enzyme, in its respective buffer as recommended by the supplier and at optimal temperature for that specific enzyme. A typical digestion includes one unit of enzyme per microgram of starting DNA. One enzyme unit is usually defined as the amount of enzyme needed to completely digest one microgram of double stranded DNA in one hour at the appropriate

temperature. Their biochemical activity of the restriction enzyme is the hydrolysis of phosphodiester backbone at specific sites in a DNA sequence. Precaution was taken to avoid star activity of restriction enzymes. When DNA is digested with certain restriction enzymes under non-standard conditions, cleavage can occur at sites different from the normal recognition sequence. Such aberrant cutting is called "star activity" which can be due to high pH (>8.0) or low ionic strength, extremely high concentration of enzyme (>100 U/µg of DNA) and presence of organic solvents in the reaction (e.g. ethanol, DMSO). The PCR and restriction digestion conditions were optimized for specific locus of relevant segment of the gene to be studied. The PCR products as well as the digested products were separated on either agarose gel or polyacrylamide gel depending on their size. Gels were stained with ethidium bromide solution (0.5 µg/ml) and subsequently visualized and photographed under UV transilluminator.

2.3.6 Detection of *ACE* (rs1799752) Polymorphism:

The angiotensin converting enzyme (ACE) is a gene situated on chromosome 17q23 that shows a 287-bp repeated Alu sequence insertion (I) or deletion (D) polymorphism in intron 16.

2.3.7 Primer sequences:

The oligonucleotides sequences (primers) used.
Forward primer - 5'-CTGGAGACCACTCCCATCCTTTCT-3'
Reverse primer - 5'-GATGTGGCCATCACATTCGTCAGAT-3'

2.3.8 PCR Mix:

For each DNA sample 25 µl of PCR reaction mixture was prepared containing 5 µl template DNA (final concentration 100-200 ng/ µl), 2.5 µl of 10X *Taq* polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; Genetix Biotech Asia Pvt. Ltd., India), 1 µl of 10 mM dNTPs (Banglore Genei, Bangalore, India), 1 µl of 25 pmol/µl of forward and reverse primers specific for IL1- β gene, 0.2 µl of 5U/ µl of *Taq* DNA polymerase (final concentration 1U; Genetix Biotech Asia Pvt. Ltd.,India) and sterile water to set up the volume of reaction mixture to 25 µl.

2.3.9 Thermal profile:

Thermal profile used for the amplification of desired segment of gene was as follows: Initial denaturation at 95˚C for 2 min and 35 cycles of denaturation at 95˚C for 1 min, annealing at 54˚C for 1 min and extension at 74˚C for 1 min, followed by final extension at 74˚C for 10 min. PCR products were separated on 2% agarose gel (2% w/v, Sigma) using a 100 bp molecular weight (MW) marker to confirm the PCR product size of 304 bp.

2.3.10 Genotyping:

The PCR products were analyzed by electrophoresis on 3% agarose gel then visualized under a UV transilluminator with 100-bp ladder. The amplification products were; 490 bp in of the I (insertion) allele and 190 bp of the D (deletion) allele

3. Results

3. 1 Detection of *ACE* (rs1799752) polymorphism:

The angiotensin converting enzyme (ACE) is a gene situated on chromosome 17q23 that shows a 287-bp repeated Alu sequence insertion (I) or deletion (D) polymorphism in intron 16. The PCR products were analyzed by electrophoresis on 3% agarose gel then visualized under a UV transilluminator with 100-bp ladder. The amplification products were; 490 bp in of the I (insertion) allele and 190 bp of the D (deletion) allele **(Depicted in figure no. 1.)** Genotype frequencies, allele frequencies and carriage rates of IL-1Ra VNTR alleles are depicted in table no. 4.6. Genotype frequency between Hypertension patient and healthy control groups were slightly different and but not significantly associated with Hypertension ($\chi 2$=2.025,P=0.3633). Thus allele frequency ($\chi 2$=0.8091, P=0.3684) and carriage rate ($\chi 2$=0.4590, P=0.4981) were also not significantly different between both case and control groups. An odds ratio of 0.7860 in Hypertension group respectively for '*II*' genotype indicated a no role in protective effect of this wild type genotype. In addition, all genotype ID and DD was also not showing protective effects.

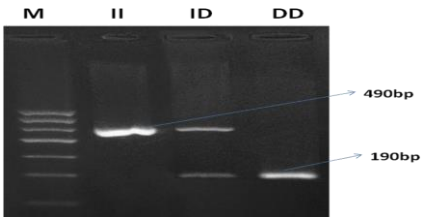


Figure -1: A representative gel picture of the *ACE* polymorphism

Data from allele frequency of allele '*I*' was found in large in the population. Here, allele frequency was not showing differences between healthy and disease population. Carriage rate of allele '*I*' was slightly high in HC group whereas carriage rate of allele '*D*' was also high in disease group but no significant association showing in carriage rate. The pattern of genotype and allele distribution in disease and control group suggested no significant association of ACE gene with Hypertension disease.

Table 1. Frequency distribution and association of Genotype, allele frequency and carriage rate of ACE gene polymorphism in population of Vindhyan region using Chi Square Test

ACE GENE	CASE N= 96		CONTROL N=112		CHI SQUARE VALUE χ^2 (P Value)
	N	%	N	%	
Genotype					
I I	42	43.75	59	52.67	
I D	48	50.00	45	40.17	2.025 (0.3633ns)
DD	06	06.25	08	07.14	
Allele					
I	132	68.75	163	72.76	
D	60	31.25	61	27.23	0.8091 (0.3684ns)
Carriage Rate					
I	90	62.50	104	66.24	
D	54	37.50	53	33.75	0.4590 (0.4981ns)

(- denotes the level of significant association between case and control.)*

(N – Number of individuals in study group.)

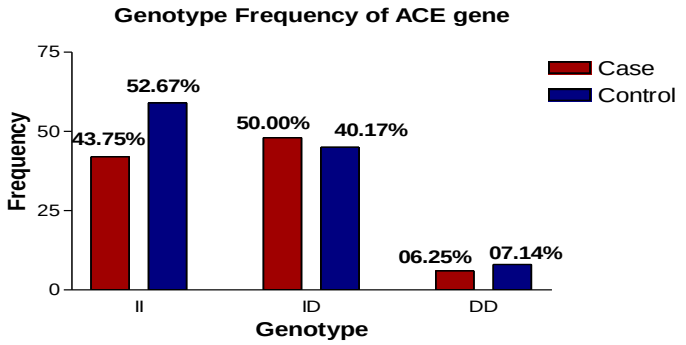
(% - Genotype allele frequency and carriage rate expressed in percentage

Table-2. Fisher Exact Test values of ACE gene polymorphism

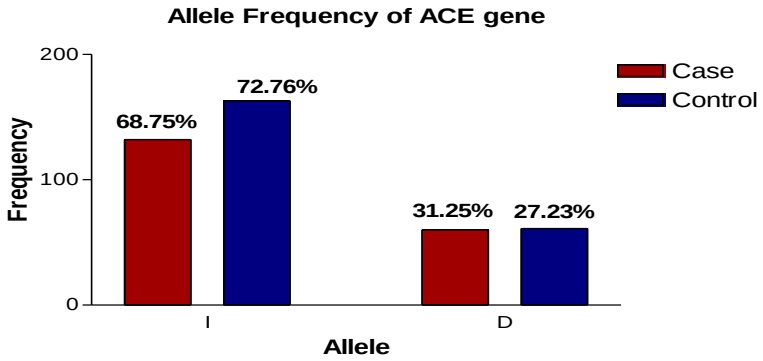
ACE GENE	CASE N= 96		CONTROL N=112		P Value	Odds Ratio (95% confidence interval)
	N	%	N	%		
Genotype						
I I	42	43.75	59	52.67	0.4792	0.7860 (0.4507 to 1.371)
I D	48	50.00	45	40.17	0.1646	1.489 (0.8587 to 2.582)
DD	06	06.25	08	07.14	1.0000ns	0.8667 (0.2897 to 2.593)
Allele						0.8233 (0.5388 to 1.258)
I	132	68.75	163	72.76		
D	60	31.25	61	27.23	0.3877	1.215 (0.7949 to 1.856)
Carriage Rate						
I	90	62.50	104	66.24		0.8494 (0.5295 to 1.363)
D	54	37.50	53	33.75	0.5472	1.177 (0.7339 to 1.889)

(- denotes the level of significant association between case and control.)*

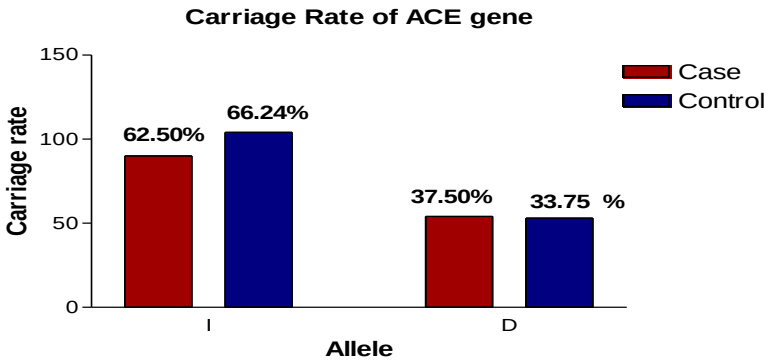
(N – Number of individuals in study group.)
(% - Genotype allele frequency and carriage rate expressed in percentage.)



Graph No.-1: Genotype Frequency of ACE gene



Graph No.-2: Allele Frequency of ACE gene



Graph No.-3: Carriage rate of ACE gene

4. Discussion

The polymorphic variant of ACE gene in intron 16 confers susceptibility to essential hypertension. I/D polymorphism at the locus intron 16 of the ACE gene were amplified from the genomic DNA of the total 571 (hypertensive patients, n: 279; controls, n: 292) participants using polymerase chain reaction and gel electrophoresis methods and were examined in a case–control approach. Suitable descriptive statistics was used for different variables. I/D polymorphism in the angiotensin-I-converting enzyme gene at the 16th intron can be useful for outcome predictions during diagnostic processes can be implicated in an individual's propensity for hypertension and thus implies that genetic variants of ACE I/D might serve as a predictor for the susceptibility to hypertension **[9-13].** Although the pathophysiological mechanism of hypertension is not fully elucidated yet, a large number of pieces of evidence have shown that genetic alterations in the renin-angiotensin-aldosterone system play a central role. However, the association of insertion/deletion polymorphism of the angiotensin-converting enzyme (ACE) gene

with essential hypertension is controversial yet, and there is a limited number of publications among the Ethiopian population. Therefore, this study aimed to determine the association of ACE gene I/D polymorphism with the risk of hypertension among essential hypertension patients. Polymorphisms of the renin-angiotensin system genes influence the pathogenesis of atherosclerosis and are connected with heart diseases **[5, 15].** Researcher explore the potential associations of ACE (I/D) and AGT (M235T) gene polymorphisms with coronary artery disease (CAD). A total of one hundred and twenty Egyptian patients (Sixty with CAD and sixty without CAD) and fifty healthy control subjects were included in the study. Genotyping of ACE (I/D) and AGT (M235T) were analyzed by the polymerase chain reaction (PCR) technique. Serum lipid profiles (total cholesterol, triglyceride, HDL-C) were measured by the enzymatic colorimetric method. This study demonstrated the contribution of ACE (I/D) and AGT (M235T) gene polymorphisms individually or in combination to the presence of CAD risk in the Egyptian population. The ACE D allele and AGT T allele may be predictive in individuals at risk of developing CAD **[14-17].**

Our data from ACE gene polymorphism revealed no association to hypertention. The angiotensin converting enzyme (ACE) is a gene situated on chromosome 17q23 that shows a 287-bp repeated Alu sequence insertion (I) or deletion (D) polymorphism in intron 16. The PCR products were analyzed by electrophoresis on 3% agarose gel then visualized under a UV transilluminator with 100-bp ladder. The amplification products were; 490 bp in of the I (insertion) allele and 190 bp of the D (deletion) allele. Genotype frequencies, allele frequencies and carriage rates of IL-1Ra VNTR alleles are depicted in table no. 4.6. Genotype frequency between Hypertension patient and healthy control groups were slightly different and but not significantly associated with Hypertension ($\chi 2$=2.025,P=0.3633). Thus allele frequency ($\chi 2$=0.8091, P=0.3684) and carriage rate ($\chi 2$=0.4590, P=0.4981) were also not significantly different between both case and control groups. An odds ratio of 0.7860 in Hypertension group respectively for '*II*' genotype indicated a no role in protective effect of this wild type genotype. In addition, all genotype ID and DD was also not showing protective effects. Data from allele frequency of allele '*I*' was found in large in the population. Here, allele frequency was not showing differences between healthy and disease population. Carriage rate of allele '*I*' was slightly high in HC group whereas carriage rate of allele '*D*' was also high in disease group but no significant association showing in carriage rate. The pattern of genotype and allele distribution in disease and control group suggested no significant association of ACE gene with Hypertension disease.

An insertion/deletion (I/D) polymorphism of the gene encoding angiotensin-I converting enzyme (ACE) is shown to have association with diabetic nephropathy. The aim of this case control study was to investigate the possible role of ACE gene in the pathogenesis of nephropathy in patients with diabetes mellitus. The study included 196 subjects (145 T2DM and 51 normal controls) T2DM were classified into 2 groups: 97 diagnosed with DN, and 48 diabetic without nephropathy as +ve control **[22,12].** Blood samples from subjects and controls were analyzed to investigate the ACE(I/D) genotypes. The homozygous genotype (DD) was significantly (OR=2.73, CI 95% 1.1- 7.3, P= 0.04) increased the risk of DN two folds with respect to those of the wild type (II) after adjustment for age, sex and BMI **[19].** Also significant variation was obtained when the analysis was carried out without adjustment (OR=3.95, CI 95% 1.7- 9.2, P= 0.001). Similarly the ID genotype significantly (OR = 3.68, CI 95%=1.5- 8.7, P= 0.03) raised the risk of DNA by three folds. Dominant and recessive models highlighted significant (P= 0.003) association of dominant model with the risk of DN which raised by three folds. the minor allele frequency (D) was significantly higher (P=0.04) in DN when compared with that of the normal control group **[18-24].**

The relationship between ACE I/D, ACE2 G8790A and CYP11B2-344T/C gene polymorphisms and essential hypertension (EH) were inconsistent. Moreover, few studies have reported the combined effect of these gene polymorphisms and noise exposure on EH. The purpose of this study was to explore the combined and separate effects of ACE I/D, ACE2 G8790A and CYP11B2-344T/C gene polymorphisms and noise on EH among steelworkers. A case–control study was conducted on 725 male workers between March 2014 and July 2014 in the Tangsteel Company, China **[26,8].** The risk of having EH for ACE I/D DD genotype carriers was 1.99 times that for II genotype carriers (95% CI 1.14–3.51). There was a negative additive interaction between ACE2 G8790A and CYP11B2-344T/C on EH (U3 = − 2.221, P = 0.026, and S = 0.128) and a positive multiplicative interaction between ACE I/D and CYP11B2 344T/C on essential hypertension (P = 0.041). In addition, there was no significant gene–noise interaction model through the GMDR method after adjusting the confounders. The ACE DD genotype may make men susceptible to EH. Simultaneously carrying the DD genotype of ACE I/D and the TC genotype of CYP11B2-344T/C increased the risk of EH **[25-28].**

5. Conclusion:

Our data from ACE gene polymorphism revealed no association to hypertention. The angiotensin converting enzyme (ACE)

6. References

1. Members WG, Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics-2008 update. Circulation. 2008;125(1):188–97.
2. Chen WW, Gao RL, Liu LS. Summary of "China Cardiovascular Disease Report 2016". Chin Circ J. 2017;32(6):521–30.
3. Zhou M, Wang H, Zhu J, Chen W, Wang L, Liu S, et al. Cause-specific mortality for 240 causes in China during 1990-2013: a systematic subnational analysis for the global burden of disease study 2013. Lancet. 2016;387(10015):251–72.
4. Sun B, Cheng X, Ma L, Tian H, Li C. Relationship between metabolic diseases and all-cause and cardiovascular disease death in elderly male diabetics during a 10-year follow-up. Zhonghua Yi Xue Za Zhi. 2014;94(8):591–5.
5. BuseJ B, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, et al. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the american heart association and the American diabetes association. Diabetes Care. 2007;30(1):162–72.
6. Bonnardeaux A, Davies E, Jeunemaitre X, Féry I, Charru A, Clauser E, et al. Angiotensin ii type 1 receptor gene the correct variation type in human essential hypertension. Hypertension. 1994;24(1):63.
7. Stanković A, Živkovic M, Glišić S, Alavantić D. Angiotensin ii type 1 receptor gene polymorphism and essential hypertension in serbian population. Clin Chim Acta. 2003;327(1):181–5.
8. Li H, Liu MY, & Wang Q. Study of relationship between ace and at1r abnormality in diabetic mellitus. Modern Preventive Medicine, 2010.
9. Lou QL, Bian RW, Xie YC, Liu-Bao GU, Ouyang XJ, Xia H, MO YZ. Study on rs5186 single nucleotide polymorphism of angiotensinIItype 1 receptor gene in type 2 diabetes complicated with hypertension. Acta Universitatis Medicinalis Nanjing. 2006;26(9):764–8.
10. Lesage S, Velho G, Vionnet N, Chatelain N, Demenais F, Passa P, et al. studies of the renin-angiotensin system in arterial hypertension associated with non-insulin-dependent diabetes mellitus. J Hypertens. 1997; 15(6):601–6.
11. Lin J, Hu FB, Qi L, Curhan GC. Genetic polymorphisms of angiotensin-2 type 1 receptor and angiotensinogen and risk of renaldysfunction and coronary heart disease in type 2 diabetes mellitus. BMC Nephrol. 2009;10:9.
12. Thameem F, Voruganti VS, He X, Nath SD, Blangero J, MacCluer JW, et al. Genetic variation in the renin-angiotensin system modifies the beneficial effects of ACE inhibitors on the risk of diabetes mellitus among hypertensives. Hum Genet. 2008;124(5):557–9.
13. Mehri S, Koubaa N, Hammami S, Mahjoub S, Chaaba R, Nakbi A, et al. Genotypic interactions of renin-angiotensin system genes with diabetes type 2 in a Tunisianpopulation. Life Sci. 2010;87(1–2):49–54.
14. Wu ZS, Zhu DL. 2010 Chinese guidelines for the management of hypertension, 2010 revision. China: In Liu LS, editor. People's Medical Publishing House. Academic; 2012. p. 1–18.
15. Wen ZM, Zhou BF, Chen JL, Li GW. Chinese guidelines for the management of adult overweight and obesity prevention and control. China: In. Chen CM, Kong LZ, editors. People's Medical Publishing House. Academic; 2006.p. 9–33.
16. Ding X, Li M, Gu H, Peng X, Zhang Z, Wu F. Detecting SNP combinations discriminating human populations from HapMap data. IEEE Tran Nanobioscience. 2015;14(2):220–8.
17. Furuta H, Guo DF, Inagami T. Molecular cloning and sequencing of the gene encoding human angiotensin ii type 1 receptor ☆. Biochemical & Biophysical Research Communications. 1992;183(1):8.
18. Doria A, Onuma T, Warram JH, Krolewski AS. Synergistic effect of angiotensin ii type 1 receptor genotype and poor glycaemic control on risk of nephropathy in iddm. Diabetologia. 1997;40(11):1293–9.
19. Saab YB, Gard PR, Overall AD. The association of hypertension with reninangiotensin system gene the correct variation type in the Lebanese population. JRAAS. 2011;12(4):588.
20. Pan G, Pu XQ, Xu XP, Feng XJ. Association of ACE and AT1R gene the correct variation type on incidence of primary hypertension and efficacy of telmisartan therapy. Chin J Biomed Eng. 2010;16(1):42–7.

21. Liu DX, Zhang YQ, Hu B, Zhang J, Zhao Q. Association of at1r polymorphism with hypertension risk: an update meta-analysis based on 28,952 subjects. J Renin-Angiotensin-Aldosterone Syst. 2015;16(4):898.
22. Wang WYS, Zee RL, Morris BJ. Association of angiotensin ii type 1 receptor gene polymorphism with essential hypertension. Clin Genet. 2010;51(1):31–4.
23. Xue YM, Zhou L, Luo R. Correlation between angiotensin ii type 1 receptor gene polymorphism and type 2 diabetes mellitus complicated by hypertension. Di Yi Jun Yi Da Xue Xue Bao. 2002;22(5):444–6.
24. Karpov RS, Puzyrev KV, Koshel'Skaia OA, Makeeva OA, Suslova TE, Efimova EV, et al. Polymorphic markers of gnb3 (c825t), agtr1 (rs5186) and ace (a2350g and i/d) genes in patients with arterial hypertension co-morbid with diabetes mellitus type 2. Terapevticheski Arkhiv. 2004;76(6):30–5.
25. Colditz GA, Willett WC, Rotnitzky A, Manson JE. Weight gain as a risk factor for clinical diabetes mellitus in women. Am J Epidemiol. 1990;132(3):501–13.
26. Lu JM. The relationship between body weight and cardiovascular mortality.Chinese J Diabetes. 2016;24(4):289–95.
27. Song X, Tabák AG, Zethelius B, Yudkin JS, Söderberg S, Laatikainen T, et al. Obesity attenuates gender differences in cardiovascular mortality. Cardiovasc Diabetol. 2014;13(1):1–10.