International Journal of Molecular Biology
ISSN: 0976-0482 & E-ISSN: 0976-0490, Volume 4, Issue 1, 2013, pp.-87-91.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000235

EVIDENCE OF ANGIOTENSIN CONVERTING ENZYME (ACE) INSERTION POLYMORPHISM IN RHEUMATOID ARTHRITIS FROM PAKISTANI PATIENTS

MOEEZ S.[1]*, IQBAL T.[2], JOHN P.[1] AND BHATTI A.[1]

[1]Department of Health Care Biotechnology, Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Islamabad- 44000, Pakistan.
[2]Department of Medicine, Shifa College of Medicine-Shifa Tameer e Millat University Pitras Bhukhari Road, Islamabad- 44000, Pakistan.
*Corresponding Author: Email- pjohn@asab.nust.edu.pk

Received: July 08, 2013; Accepted: August 01, 2013

Abstract-

Background: The study was aimed to explore the association of I/D polymorphism genotypes in Angiotensin converting enzyme (ACE) gene in Rheumatoid Arthritis (RA) patients from the Pakistani population.

Methodology: The intron 16 of ACE gene was amplified in 200 RA patients and 200 normal healthy individuals by using a forward (ACE-F: 5´CTGGAGACCACTCCCATCCTTTCT3´) and a reverse primer (ACE-R: 5´GATGTGGCCATCACATTCGTCAGAT3´). PCR products were directly sequenced in an ABI prism 310 Automated Sequencer (Applied Biosystem). The data was statistically analyzed through SPSS software and association was tested through Chi-square and Z-test.

Results: We noticed that genotype II was most frequently occur (p <0.001) in cases as compare to our healthy controls. No significant association was analyzed between the homozygote (DD) and heterozygote (ID) genotypes and pathogenesis of RA. ACE I allele carriers were at higher risk to develop RA than those who were D carriers. An association of ACE I/D gene polymorphism was established with migratory arthritis (p >0.001).

Conclusion: Our present study identified an association between ACE insertion (II) polymorphism and Rheumatoid arthritis in the Pakistani population.

Keywords- Angiotensin-converting enzyme (ACE), Rheumatoid Arthritis, I/D Polymorphism, Primers, SNP, DNA, Pakistan, Genotype

Running Title: ACE gene polymorphism in RA

Citation: Moeez S., et al. (2013) Evidence of Angiotensin Converting Enzyme (ACE) Insertion Polymorphism in Rheumatoid Arthritis from Pakistani Patients. International Journal of Molecular Biology, ISSN: 0976-0482 & E-ISSN: 0976-0490, Volume 4, Issue 1, pp.-87-91.

Introduction

Rheumatoid arthritis (RA) is a systematic, inflammatory joints disease, occurs as a chronic inflammatory disorder that is characterized by cartilage destruction. Worldwide, its prevalence is about 0.3 -to 1.2% [1]. The disease is mainly characterized by angiogenesis of the synovium, growth of new blood vessels and infiltration of leukocytes. These events ultimately lead to invasion of tissue and destruction of joints [2]. Cytokines are thought to play an important role in the progression of Rheumatoid arthritis [3].

Exact etiology of RA is not so far known, however its development depends upon interaction between genetics elements in individual and non-genetic factors. Considerable amount of data have suggested that autoimmunity markers and genetic factors are very good indicators [4]. The contributions of genetic factors are estimated to be 50-60% [5]. Human leukocyte antigen (HLA) has the most prominent genetic association, which is estimated to be 30% of the total genetic contributions. It is also suggested that a number of non -HLA genes also play a very important role in the progression and pathogenesis of RA [6].

The angiotensin-converting enzyme (ACE) is a membrane- bound, zinc metalloendopeptidase. It is also known as peptidyl dipeptidase A or kininase II), encoded by the ACE gene. It is mapped on chromosome 17q23 and plays a central role in a number of diseases. This is a 21 kilo bases (kb) long gene having 26 exons and 25 introns [7]. ACE gene encodes two isoenzymes, the germinal and somatic. The germinal isoenzyme is expressed only in sperm, whereas somatic isoenzyme is expressed in numerous tissues, including vascular endothelial cells, testicular Leydig cells, lung and epithelial kidney [8].

In the National Center for Biotechnology Information (NCBI) records, more than 160 ACE gene polymorphisms have been reported so far, most of which are single nucleotide polymorphisms (SNPs). Only 34 of those polymorphisms are located in coding regions and 18 of them are missense mutations [7]. ACE gene contains a poly-

morphism that is based on the presence (insertion=I) or absence (deletion=D) of a 287 base pair ALU repeat sequence in intron 16; that results a 3 genotypes: DD and II homozygous and ID heterozygous [9]. The DD genotype serum level is found to be the highest in normal individuals that is followed by the heterozygous ID genotype, while the level of II genotype is lowest [10,11].

Correlation between ACE gene level and I/D polymorphism raised question whether I/D polymorphism by itself has a functional significance or it is linked to a yet unidentified molecular variant in the ACE gene or in another locus on the chromosome 17 region where ACE is mapped. It has been proposed that regulatory elements that are present in intron 16 may affect gene transcription, thus control ACE gene level in tissues [7,12].

ACE gene I/D polymorphism have been reported in different ethnic groups in different diseases including rheumatoid arthritis [Table-1]. ACE gene is a key regulator in inflammatory signal transduction pathways and is involve in RA pathogenesis [13]. Level of ACE gene in serum and tissue is regulated at transcriptional level and it can be hypothesized that that ACE genotype may be a contributing factor in the onset, pathogenesis and progression of RA [6].

Table 1- Study of ACE gene polymorphism in different diseases

SN	Disease	Population	Year	Result
1	Psoriatic arthritis	Kuwait	2007	Not Associated
2	Cardiovascular disease	Italy	2012	Associated
3	Type 2 diabetes	Indonesia	2010	Not Associated
4	Rheumatoid arthritis	Kuwait	2007	Associated
5	Alzheimer Disease	Israeli Arab Community	2006	Associated
6	Systemic Lupus Erythematosus	Pakistan	2010	Associated
7	Atherosclerosis	Asians	2003	Associated
8	Breast cancer	Caucasian women	2005	Associated
9	Rheumatoid Arthritis (**This Work**)	Pakistan	2013	Associated

To the best of our knowledge, the possible role of this polymorphism to control risk to develop RA has not been studied in the Pakistani population. Therefore, to study the functional allelic differences in the ACE I/D polymorphism may be a determining factor in the pathogenesis of RA.

Materials and Methods

Materials

dNTp's (Invitrogen), Taq polymerase (Invitrogen), Mgcl2 (Fermantas), DNA ladder (Fermentas) and Agrose (Invitrogen) were used in this study. PCR Primers were designed using Primer 3 input software version 0.40 and were BLAST using NCBI PRIMER BLAST. Primer sequences were obtained from Eurofins Scientific.

Blood Collection and Storage of Samples

Blood samples were collected from October 2011 to June 2012 with a prior approval from Ethical Committees and Institutional Review Boards (IRBs) of National University of Sciences and Technology and the hospitals. All the patients fulfilled the American College of Rheumatology classification criteria. Informed written consent was obtained from each patient. The subjects were classified into different groups on the basis of age, gender and severity of disease (mild, moderate and severe). Two Hundred RA Patients (mean age 41±13) & two hundred healthy control individuals (mean age 42±12) were recruited in the present study. Each blood sample was collected in EDTA tube and was kept at 4°C.

DNA Isolation

DNA was extracted from blood of RA patients and healthy individuals by using standard Phenol-Chloroform method and the DNA was quantified through nanodrop.

Polymerase Chain Reaction

The intron 16 of ACE gene was amplified in a total reaction volume of 20µL. Each reaction volume contained 10 pmol of each forward and reverse primer: ACE-F: 5´CTGGAGACCACTCCCATCCTTTC T3´ and ACE-R: 5´GATGTGGCCATCACATTCGTCAGAT3´, 25mM $MgCl_2$, 100mM of 10x Taq buffer, 10mM dNTPs, 1U Taq polymerase and 2µL of DNA. Reaction conditions were 96°C for 5 minutes; 94°C for 30 seconds; 58°C for 1 minute, 72°C for 1 minute and a final extension at 72°C for 10 minutes. The PCR products which were amplified further run on 2% agarose gel using 100bp DNA ladder as a marker.

Hardy-Weinberg Equilibrium and Genotypes Distribution

Genotype frequencies in cases and controls were calculated according to Hardy-Weinberg equilibrium. This law states that: in population both allele and genotype frequencies remains constant, means they are in equilibrium from generation to generation unless specific disturbing influences are introduced.

Sequencing

PCR products were then sequenced directly in an ABI prism 310 Automated Sequencer, using the ABI Big dye Terminator V 3.1 cycle sequencing kit (PE Applied Biosystem), following purification by Rapid PCR Purification System (Marligen Biosciences USA).

Statistical Analysis

Statistical analysis was performed using SPSS v 16.0 using the level of $p \leq 0.05$ as statistically significant. Alleles and genotype frequencies between patients and control subjects were compared by Z-test. Some clinical characteristics of patients and Hardy-Weinberg equilibrium were analyzed by Chi-Square analysis.

Results

Association analysis of ACE gene I/D polymorphism was carried out in two hundred RA patients and two hundred healthy individuals using allele specific PCR as shown in [Fig-1] & [Fig-2]. Significant difference was found in frequencies of ACE genotypes and I/D alleles between patients and controls ($p < 0.001$).

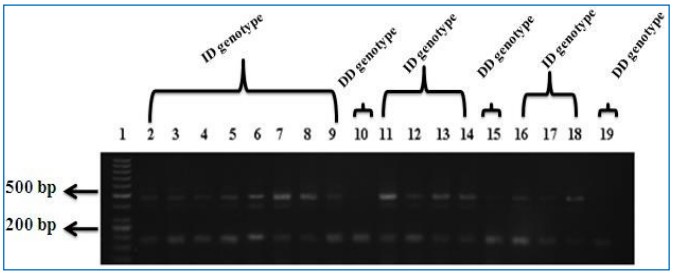

Fig. 1- Detection of ACE gene I/D polymorphism by polymerase chain reaction (PCR). *Lane 1:* 100 bp DNA ladder, *Lanes 2-9:* PCR products from patients with ID genotype, *Lane 10:* PCR product from patient with DD genotype, *Lanes 11-14:* PCR products from patients with ID genotype, *Lane 15:* PCR product from patient with DD genotype, *Lanes 16-18:* PCR products from patients with ID genotype and *Lane 19:* PCR product from patients with DD. The products of PCR amplification were analyzed on 2% agarose gel and visualized under UV light.

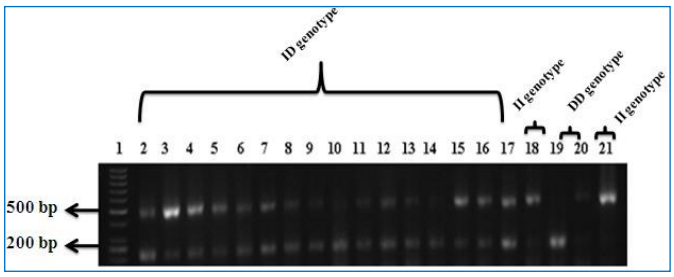

Fig. 2- Detection of ACE gene I/D polymorphism by polymerase chain reaction (PCR). *Lane 1:* 100 bp DNA ladder, *Lanes 2-17:* PCR products from patients with ID genotype, *Lane 18:* PCR product from patient with II genotype, *Lanes 19:* PCR product from patients with DD genotype, *Lane 20:* PCR product from patient with ID genotype and *Lane 21:* PCR product from patients with II genotype, lane 6 PCR products from patients with II genotype. The products of PCR amplification were analyzed on 2% agarose gel and visualized under UV light.

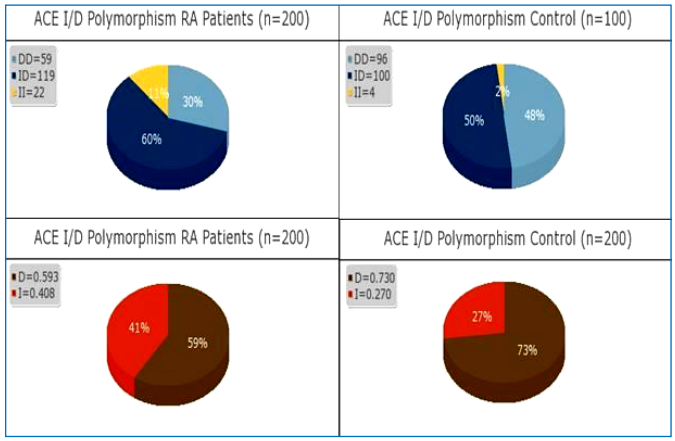

Fig. 3- Distribution of the study subjects across different genotypes for the studied polymorphisms.

Table 2- ACE allele and genotype frequencies with respect to patient characteristics in the RA

RA Patient Characteristics	n=200	Genotype Frequency			Allele Frequency		*p* Value
		DD	II	ID	I	D	
Gender							
Male	87	18	13	56	0.47	0.53	0.068
Female	113	34	7	72	0.38	0.62	
Age at onset							
≤40	135	35	9	91	0.4	0.6	0.272
>40	65	17	12	36	0.47	0.53	
Family history							
Yes	31	18	2	11	0.24	0.76	*0.001
No	169	34	18	117	0.46	0.54	
Duration of symptoms							
>6weeks	54	15	5	34	0.4	0.6	
>6months	90	24	6	60	0.4	0.6	0.53
>1year	56	13	9	34	0.47	0.53	
Migratory Arthritis							
Yes	104	26	10	68	0.58	0.42	*0.01
No	96	26	10	60	0.6	0.4	
Disease Severity							
Minor	32	12	2	18	0.34	0.66	
Moderate	117	26	15	76	0.46	0.54	0.15
Severe	51	14	3	34	0.4	0.6	

We identified a fragment of 490bp which shows an insertion of Data regarding ACE gene I/D polymorphism genotypes frequencies in cases and controls are presented in [Fig-3]. In addition when a comparison was made between ACE genotype sub groups including migratory arthritis and familial type cases then significant association was observed ($p < 0.001$). However no significant association was found with gender, age at onset, duration of symptoms and disease severity ($p > 0.05$) [Table-2]. ACE genotype distribution was in accordance with the Hardy-Weinberg expectations in the cases and control group.

289bp sequences when compared with wild type fragment of 190bp [Fig-4]. The 2% agarose gel result was confirmed by sequencing [Fig-5]. This sequence was aligned with the control using EMBOSS pair wise alignment tool, which showed 100% homology except the 289bp insertion. The mutant observed in this study is reported previously [14].

CTGGAGACCACTCCCATCCTTTCTCCCATTTTTCTAGACCTGCTGCCTATACAGTC
TCTTTTTTTTTTTTTTGAGACGGAGTCTCGCTCTGTCGCCCAGGCTGGAGTGC
AGTGGCGGGATCTCGGCTCACTGCAAGCTCCGCCTCCCGGGTTCACGCCATTCT
CCTGCCTCAGCCTCCCAAGTAGCTGGGACCACAGGCGCCCGCCACTACGCCCG
GCTAATTTTTTGTATTTTTAGTAGAGACGGGGTTTCACCGTTTTAGCCGGGATG
GTCTCGATCTCCTGACCTCGTGATCCGCCCGCCTTGGCCTCCCAAAGTGCTGGG
ATCACAGGCGTGATACAGTCACTTTTATGTGGTTTCGCCAATTTTATTCCAGCTC
TGAAATTCTCCGAGCTCCCCTTACAAGCAGAGGTGAGCTAAGGGCTGGAGCTC
AAGGCATTCAAACCCCTACCAG ATCTGACGAATGTGATGGCCACGTC

Fig. 4- The 289 bp Sequence in intron 16 of ACE gene. Forward & Reverse Primers (Blue) 289bp insertion (Red) with intronic sequence (Black).

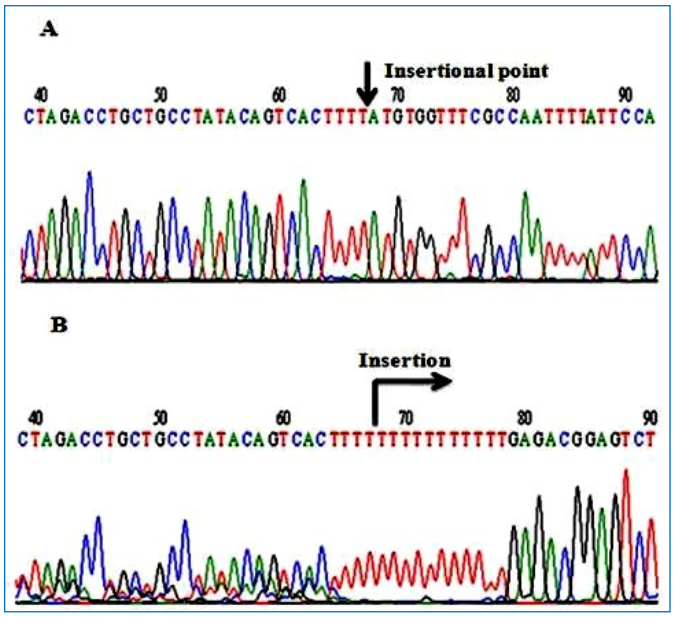

Fig. 5- Sequencing Electropherogram showing analysis of ACE gene polymorphism **(A)** Sequence analysis of DD genotype of ACE gene in control sample with arrow indicating the point of insertion. **(B)** Insertion of 289bp in II genotype polymorphism of ACE gene in RA patients.

Discussion

Rheumatoid arthritis is an inflammatory disease and various cytokines are involved in the regulation of key pathways during disease pathogenesis. It is an autoimmune disease with complex etiology. Various hormonal, environmental and genetic factors have signifi-

cant role in the onset of RA. Considerable amount of data have suggested that autoimmunity markers and genetic factors are very good indicators in RA. About 30%, HLA genes are involved whereas other non-HLA genes may also involve in RA progression [6]. To understand the RA genetic susceptibility from a different point, RA patients are supposed to share few pathogenic mechanisms that may contribute in inflammation [15,16]. As RA is an environmental based disease, so its occurrence varies from population to population. I/D polymorphism in intron 16 of ACE gene was first observed when it was reported that it accounts for about half of the observed variance in plasma levels. The position of this polymorphism in a non-coding region of the gene however; makes it unlikely to be a functional variant [7,17]. This polymorphism has been studied in different population as shown in [Table-1]. So far, it has not been studied in Pakistani population.

In the present study, we studied the distribution of I/D polymorphism genotypes of ACE gene in RA patients in Pakistan to evaluate its potential role in RA pathogenesis. The present study indicates that the percentage of ACE gene I/D polymorphism allele and the distribution of genotypes are not significantly different between patients and controls except II. So far, there are only some studies have been published regarding to ACE gene I/D polymorphism in RA. Consistent with our study Alsaeid, et al [18]. has found a significantly higher incidence of II genotype was observed in the juvenile idiopathic arthritis patients [18]. However, in contrast Uppal etal found a significant overrepresentation of the DD genotype and the D allele in patients with RA [6].

Systemic lupus erythematosus (SLE), is one more multisystem autoimmune disease, has also been revealed to be linked with ACE gene polymorphism. The DD genotype was more common in SLE patients than healthy controls [19]. A large difference has been reported in the usual circulation of ACE gene alleles in different populations. The frequency of D allele in normal Caucasians is 50-58%, but 35-39% in normal Chinese [20-22].

The renin-angiotensin system plays an key role in maintaining body fluid and sodium balance and has been involved in a many complex disorders [23-25]. ACE gene involves in the renin-angiotensin and kallikrein-kininogen systems by converting angiotensin I into pressor peptide angiotensin II by removing terminal His-Leu, thus inactivates vasodilator peptide bradykinin. Angiotensin II has been shown to be involved in a cascade of events through the angiotensin type-1 receptor and these actions include vasoconstriction, proliferation, hypertrophy, matrix deposition, and stimulation of growth factors [26]. Lower activity of ACE gene might be associated with the allele I of ACE gene. On the basis of this we can hypothesize that I/D polymorphism reduce levels of ACE gene in joints or in plasma which eventually increase the level of bradykinin thus activate another pathway that would cause joint inflammation in rheumatoid arthritis patients [27]. As this polymorphism is present within intron, it is assumed that it can act as a neutral marker in strong linkage disequilibrium with 1 or more unknown functional variants that are present in close vicinity of ACE gene [28,29].

There are number of studies that investigated the associations of I/D polymorphism with different pathophysiological conditions. No correlation was found to be associated with I/D polymorphim of ACE gene with psoriatic arthritis [30]. Küçükarabac, et al [31] did not reveal any difference in the ACE gene I/D polymorphism genotypes distribution between dilated cardiomyopathy patients and healthy controls. Gunes, et al [32] also did not found any difference in ACE gene I/D polymorphism genotypes in hypertensive patients and healthy controls in the Turkis population. Bayram, et al [22] reported that DD homozygotes was found to be significantly higher in osteoarthritis than in healthy controls (DD) and D allele frequency and DD homozygosis were found to be significantly higher in polycystic ovary syndrome patients than in controls in the Turkish population.

ACE gene also considered as a top candidate gene for cardiovascular studies as well [33]. There are number of studies that have shown a positive association of DD genotype and increased risk to develop myocardial infarction [34]. It has been reported previously that the D allele behaves as an indicator of atherosclerotic cardiovascular complications [35]. Thus, by considering all the data we can suggest that the DD genotype negatively influences specific cardiovascular diseases. Considering the association between RA and different cardiovascular diseases, we can suggest that is it possible that the same pathway (ACE pathway) and the same genotype (DD) are involved in both diseases [36-38].

To our knowledge this is the first molecular based study from Pakistani population, however more data is required to study the disease pathogenesis. In conclusion, many studies have described that different genes play a crucial role in the etiology of RA. Due to the limited research about role of ACE gene in RA, the current study provides an significant contribution to the literature. Our results suggest that, presence of I allele of the ACE gene polymorphism may constitute a risk for developing RA. Further work is required to confirm these findings in different study groups.

Acknowledgements

We wish to thank patients for their participation and cooperation in this study. The work was funded by The Higher Education Commission (HEC), Islamabad, Pakistan.

Conflict of Interest

This study has no conflict of interests. All authors have seen the original manuscript and they have approved it.

References

[1] Carmona L., Villaverde V., Hernández-García C., Ballina J., Gabriel R. and Laffon A. (2002) *Rheumatology*, 41(1), 88-95.

[2] Szekanecz Z. and Koch A.E. (2008) *Arthritis Res. Ther.*, 10(5), 224.

[3] Brennan F.M. and Mclnnes I.B. (2008) *J. Clin. Invest.*, 118(11), 3537-45.

[4] Klareskog L., Widhe M., Hermansson M. and Ronnelid J. (2008) *Curr. Opin. Rheumatol.*, 20 (3), 300-5.

[5] Kurreenan F.A., Padyukov L., Marques R.B., Schrodi S.J., Seddighzadeh M., Stoeken-Rijsbergen G., Annette H.M. van der Helm-van Mil, Allaart C.F., Verduyn W., Houwing-Duistermaat J., Alfredsson L., Begovich A.B., Klareskog L., Huizinga T.W.J., Rene E.M. (2007) *PLoS Med.*, 4(9), e278.

[6] Uppal S.S., Haider M.Z., Hayat S.J., Agraham M., Sukumaran J. and Dhaunsi G.S. (2007) *J. Rheumatol.*, 34, 2395-9.

[7] Sayed-Tabatabaei F.A., Oostra B.A., Isaacs A., van Duijn C.M. and Witteman J.C.M. (2006) *Circ. Res.*, 98, 1123-1133.

[8] Sabbagh A., Otrock Z.K., Mahfoud Z.R. (2007) *Mol. Biol. Rep.*, 34(1), 47-52.

[9] Turgut S., Akın F., Akcılar R., Ayada C. and Turgut G. (2011)

Mol. Biol. Rep., 38(1), 569-576.

[10]Pullmann R., Lukac JR., Skerenova M., Rovensky J., Hybenová J., Melus V., Celec S. and Hyrdel R. (1999) *Clin. Exp. Rheumatol.*, 17, 593-6.

[11]Papadopoulos K.I., Melander O., Orho-Melander M., Groop L.C., Carlsson M. and Hallengren B. (2000) *J. Intern. Med.*, 247 (1), 71-77.

[12]Rosatto N., Pontremoli R., De Ferrari G. and Ravazzolo R. (1999) *Nephrology Dialysis Transplantation*, 14(4), 868-71.

[13]Soderblom T., Nyberg P., Pettersson T., Klockars M. and Riska H. (1996) *Respiration*, 63, 272-276.

[14]Bolli P., Sticchi E., Giusti B., Saracini C., Abbate R. and Fatini C. (2010) *Journal of the Renin-Angiotensin- Aldosterone System*, 12(3), 129-132.

[15]Droge W. (2002) *Physiol. Rev.*, 82, 47-95.

[16]McCulloch C.A., Downey G.P., Gabalawy H. (2006) *Nat. Rev. Drug. Discov.*, 5, 864-76.

[17]Rigat B., Hubert C., Alhenc-Gelas F., Cambien F., Corvol P. and Soubrier F. (1990) *J. Clin. Invest.*, 86, 1343-1346.

[18]Alsaeıd K., Haıder M.Z., Ayoub E.M. (2003) *J. Rheumatol.*, 30, 2705-2709.

[19]Prkacin I., Novak B., Sertic J. and Mrzljak A. (2001) *Acta. Med. Croatica.*, 55, 73-76.

[20]Hong S.J., Yang H.I., Yoo M.C., In C.S., Yim S.V., Jin S.Y., Choe B.K., Chung J.H. (2003) *Exp. Mol. Med.*, 35 (3), 189-195.

[21]Shehab D.K., Al-Jarallah K.F., Alawadhi A.M., Al-Herz A., Nahar I. and Haider M.Z. (2008) *Clin. Exp. Rheumatol.*, 26, 305-310.

[22]Bayram B., Sayın E., Güneş H.V., Değirmenci İ., Türkoğlu Z., Doganer F., Coşan D.T. (2011) *Molecular Biology Reports*, 38 (3), 1713-1716.

[23]Rigat B., Hubert C., Alhenc-Gelas F. (1990) *J. Clin. Invest.*, 86, 1343-6.

[24]Samani N.J., Thompson J.R., O'Toole L., Channer K. and Woods K.L. (1996) *Circulation*, 94, 708-12.

[25]Webb D.J. and Gonias S.L. (1998) *Lab. Invest.*, 78, 939-48.

[26]Brock J.W., Hunley T.E., Adams M.C. and Kon V. (1998) *J. Urol.*, 160, 1812-9.

[27]Murphey L.J., Gainer J.V., Vaughan D.E. and Brown N.J. (2000) *Circulation*, 102, 829-832.

[28]Tiret L., Rigat B., Visvikis S., Breda C., Corvol P., Cambien F., Soubrier F. (1992) *Am. J. Hum. Genet.*, 51, 197-205.

[29]Villard E., Tiret L., Visvikis S., Rakotovao R., Cambien F. and Soubrier F.V. (1996) *Am. J. Hum. Genet.*, 58, 1268 -1278.

[30]Al-Awadhi A.M., Hasan A.E., Sharma P.N., Haider M.Z. and Al-Saeid K. (2007) *Rheumatol. Int.*, 27, 1119-1123.

[31]Küįükarabac B., Birdane A., Günefl H.V., Ata N. (2008) *Anadolu Kardiyol Derg*, 8(1), 65-66.

[32]Gunes H.V., Ata N., Degirmenci I., Basaran A., Timuralp B., Dikmen M., Ustuner C., Kudaiberdieva G. (2004) *Int. J. Clin. Pract.*, 58(9), 838-843.

[33]Mayer B. and Schunkert H. (2000) *Herz*, 25, 1-6.

[34]Niu T., Chen X., Xu X. (2002) *Drugs*, 62, 977-93.

[35]Staessen J.A., Wang J.G., Ginocchio G., Petrov V., Saavedra, A.P., Soubrier F., Vlietinck R., Fagard R. (1997) *J. Hypertens.*, 15, 1579-92.

[36]Chung C.P., Avalos I., Raggi P., Stein C.M. (2007) *Clin. Rheumatol.*, 26, 1228-33.

[37]Chung C.P., Oeser A., Solus J.F., Avalos I., Gebretsadik T., Shintani A., Raggi P., Sokka T., Pincus T., Stein C.M. (2008) *Atherosclerosis*, *196*(2), 756-763.

[38]del Rincón I., O'Leary D.H., Freeman G.L., Escalante A. (2007) *Atherosclerosis*, 195(2), 354-360.