Significant Association of Insertion/Deletion Polymorphism of the Angiotensin-Converting Enzyme Gene with Rheumatoid Arthritis

SUKHBIR S. UPPAL, MOHAMMAD Z. HAIDER, SAWSAN J. HAYAT, MINI ABRAHAM, JALAJA SUKUMARAN, and GURSEV S. DHAUNSI

ABSTRACT. ***Objective.*** Only 30% of the genetic contribution to rheumatoid arthritis (RA) can be attributed to HLA genes, and other non-HLA genes may play a role in RA susceptibility. Angiotensin-converting enzyme (ACE) has been reported to be involved in pathogenesis of RA, and high levels of ACE have been documented in RA synovial fluid and pleural effusions. Since plasma and tissue levels of ACE are determined at the transcriptional level, we test the hypothesis that the genotype of ACE in RA patients may be a determining factor in pathogenesis.

Methods. Sixty patients with RA were recruited and clinically characterized according to disease duration, disease severity, disease activity, and American College of Rheumatology functional classes. ACE gene I/D polymorphism genotypes were determined in patients and healthy controls, using polymerase chain reaction.

Results. We found a significant overrepresentation of the DD genotype and the D allele in patients with RA; and we found that men with RA exhibited a higher frequency of the DD genotype and D allele compared to male controls. By logistic regression analysis the DD genotype confers a relative risk for development of RA of 3.

Conclusion. Our study found an association between ACE deletion polymorphism and RA. (First Release Nov 1 2007; J Rheumatol 2007;34:2395–9)

Key Indexing Terms:
ANGIOTENSIN-CONVERTING ENZYME KUWAIT RHEUMATOID ARTHRITIS
INSERTION/DELETION POLYMORPHISM

The renaissance in the study of the genetic susceptibility to rheumatoid arthritis (RA), the commonest chronic inflammatory polyarthritis seen in rheumatology practice, is important in view of its diagnostic and therapeutic potentials. Only 30% of the genetic contribution to RA can be attributed to HLA genes, and it is suggested that other non-HLA genes play a role in RA susceptibility[1]. Patients with RA are believed to share some common pathogenic mechanisms that involve inflammation and elaboration of reactive oxygen species[2,3]. Recently, angiotensin-converting enzyme (ACE), a key regulator in inflammatory signal transduction pathways, has been reported to be involved in pathogenesis of RA, and high levels of ACE have been documented in RA synovial fluid[4,5] and RA pleural effusions[6]. An insertion (I)/deletion (D) polymorphism of a 287 base-pair fragment in intron 16 of the ACE gene has been found to account for 47% of the total variance of plasma ACE activity[7]. It has been confirmed that the serum ACE levels correspond to the order II < ID < DD genotypes in healthy individuals[8,9]. Since plasma and tissue levels of ACE are determined at the transcriptional level, we hypothesized that the genotype of ACE gene in patients with RA may be a determining factor in the onset and pathogenesis of this inflammatory disease.

From the Departments of Medicine and Pediatrics, Faculty of Medicine, Kuwait University; and Department of Medicine, Mubarak Al-Kabeer Hospital, Kuwait.

S.S. Uppal, MBBS, MD, FICP, FRCP Edin, Associate Professor, Consultant Rheumatologist, Departments of Medicine, Kuwait University and Mubarak Al-Kabeer Hospital; M.Z. Haider, PhD, Professor; J. Sukumaran, MSc, Scientific Assistant; G.S. Dhaunsi, PhD, Associate Professor, Department of Pediatrics; M. Abraham, MBBS, Laboratory Physician, Department of Medicine, Kuwait University; S.J. Hayat, BMBCh, Registrar in Medicine, Department of Medicine, Mubarak Al-Kabeer Hospital.

Address reprint requests to Dr. S.S. Uppal, Department of Medicine, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait. E-mail: uppalss@hsc.edu.kw

Accepted for publication July 5, 2007.

MATERIALS AND METHODS

Patient recruitment and evaluation. Sixty consecutive patients with RA attending the Rheumatology Clinic at Mubarak Al-Kabeer Hospital, Kuwait, were recruited and ACE gene I/D polymorphism genotypes were determined in them. All patients fulfilled the American College of Rheumatology (ACR) classification criteria for RA[10] and gave informed consent for the study. A complete clinical evaluation was done for all patients. Standardized joint counts and patient ratings of general health on a 100 mm visual analog scale were recorded. Data were entered into a predesigned proforma. The 4-variable Disease Activity Score 28 (DAS28-4)[11] was computed using the formula: $0.56 \times \sqrt{(28TJC)} + 0.28 \times \sqrt{(28SJC)} + 0.70 \times \ln(ESR) + 0.014 \times GH$, where TJC = tender joint count, SJC = swollen joint count, ESR = erythrocyte sedimentation rate, and GH = general health. A cutoff point of < 2.6 was used for

the DAS28-4 to define remission, corresponding with fulfillment of the modified American Rheumatism Association criteria for remission[12,13].

The patients were clinically characterized on the basis of disease duration into having early (< 3 mo), established (> 3 mo to up to 2 yrs), or late (> 2 yrs) disease[14,15]; on the basis of maximum number of joints involved at any stage into mild (3–6 joints), moderate (> 6 but < 20 joints), or severe (> 20 joints) disease[15,16]; on the basis of assessed disability into American College of Rheumatology (ACR) functional class I, II, III, or IV; and on the basis of DAS28-4 as being in remission (< 2.6), or being active (> 2.6)[17].

Treatment strategy for patients with RA followed guidelines published recently[18]. All patients were treated with disease modifying antirheumatic drugs (DMARD) and/or biologicals, and whenever appropriate nonsteroidal antiinflammatory drugs (NSAID) and/or corticosteroids were added.

Thirty-five healthy controls were also enrolled in the study.

Genotyping. Blood samples were collected from patients and controls. Total genomic DNA was isolated by a standard procedure[19]. ACE genotypes were determined by polymerase chain reaction (PCR) using primers and conditions described previously[7]. Reactions were performed with 10 pmol of each primer: sense oligo: 5' CTG GAG ACC ACT CCC ATC CTT TCT 3' and antisense oligo: 5' GAT GTG GCC ATC ACA TTC GTC AGA T 3' in a final volume of 50 μl, containing 3 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl pH 8.4, 0.1 mg/ml gelatin, 0.5 mM of each dNTP (Cetus), 2.5 u AmpliTaq DNA polymerase (Cetus). DNA was amplified for 30 cycles with denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min using a Perkin-Elmer thermal cycler. 0.6% dimethylsulfoxide is routinely added to the PCR mix in order to improve the amplification of the I allele and avoid mistyping it as a D allele. PCR products were analyzed on 2% agarose gels after staining with ethidium bromide. In the absence of the 287 bp insertion in intron 16 of the ACE gene, this PCR method resulted in a 190 bp product (D allele) and in the presence of insertion, produced a 490 bp product (I allele). In heterozygous samples, 2 bands (490 and 190 bp) were detected along with a third fragment of intermediate size, which corresponds to a heteroduplex DNA fragment[20]. In all PCR analyses, appropriate positive and negative controls were included.

Statistical methods. Statistical analysis was performed using SPSS v 12.0 (SPSS Inc., Chicago, IL, USA) using the level of $p \leq 0.05$ as statistically significant. Chi-square test and Fisher's exact were used to assess the association between 2 qualitative variables. Kruskal-Wallis test was used to correlate gene polymorphism and age at onset in patients with RA. Logistic regression was used to estimate the risk of factors in RA after controlling confounding between them. The variables sex and ethnicity were included in the model to control for confounding. The adjusted odds ratios (OR) and their 95% confidence intervals (CI) for associated factors were computed from the coefficients of the conditional logistic regression model. The allele and genotype frequencies followed normal Hardy-Weinberg distribution in our population.

RESULTS

Genotype and allele frequencies subgrouped by sex and ethnicity are presented in Tables 1 and 2.

First, the ACE genotype frequencies were delineated. A significant difference was found in ACE I/D genotype frequencies between patients and controls (p = 0.014), with an overrepresentation of the DD genotype in patients compared to controls. In addition, when a comparison was made between the demographic subgroups of patients with RA, it was noted that sex correlated significantly with genotypic frequencies. Importantly, male patients with RA exhibited a higher frequency of the DD genotype compared to male controls. In contrast, when Arab patients were compared to non-Arabs, ethnicity did not translate into any significant differences in ACE genotypes.

Next, the frequencies of ACE alleles were delineated. A significant difference was found in frequencies of ACE I/D alleles between patients and controls, with RA patients having a higher representation of D and lower representation of I alleles compared to controls. When comparing frequencies of ACE I/D alleles between each subgroup of patients and controls, a higher frequency of D allele was found in male patients with RA compared to male controls, and Arab patients showed a similar higher frequency of D allele compared to Arab controls. No such difference could be found in non-Arabs.

Next, when a comparison was made between the subgroups of patients with RA as per disease variables (Table 3), a significant correlation was observed between ACR functional class of patients with RA and ACE genotypes (p = 0.035). Additionally, although the frequency of D allele was higher in patients with severe disease (22.5% vs 12.9%) compared to controls, these figures did not reach statistical significance. However, no correlation was observed for DAS28 scores, TJC, SJC, ESR, disease duration, or requirement for DMARD with regard to frequency of ACE genotypes or alleles (data not shown).

Finally, logistic regression analysis was used to adjust confounding between demographic factors. Table 4 presents the

Table 1. Angiotensin-converting enzyme (ACE) allele and genotype frequencies in patients with RA and healthy controls.

Variable	Male			Female			All		
	Controls, n = 14 n (%)	Patients, n = 19 n (%)	p	Controls, n = 21 n (%)	Patients, n = 41 n (%)	p	Controls, n = 35 n (%)	Patients, n = 60 n (%)	p
ACE genotype frequency			0.031[b]*			0.085[b]			0.014[a]*
II	8 (57.1)	6 (31.6)		2 (9.5)	4 (9.7)		10 (28.6)	10 (16.7)	
ID	4 (28.6)	2 (10.5)		9 (42.9)	9 (22.0)		13 (37.1)	11 (18.3)	
DD	2 (14.3)	11 (57.9)		10 (47.6)	28 (68.3)		12 (34.3)	39 (35.0)	
ACE allele frequency			0.006[a]*			0.208			0.003[a]*
I	20 (71.4)	14 (36.8)		13 (31.0)	17 (20.7)		33 (47.1)	31 (26.8)	
D	8 (28.6)	24 (63.2)		29 (69.0)	65 (79.3)		37 (52.9)	89 (74.2)	

p values calculated by [a] chi-square test and [b] Fisher's exact test. * Values are significant.

Table 2. Angiotensin-converting enzyme (ACE) allele and genotype frequencies in patients with RA and control subjects according to ethnicity.

Variable	Arabs			Non-Arabs		
	Controls, n = 20 n (%)	Patients, n = 36 n (%)	p	Controls, n = 15 n (%)	Patients, n = 24 n (%)	p
ACE			0.082[b]			0.158[a]
II	4 (20.0)	3 (8.3)		6 (40.0)	7 (29.2)	
ID	7 (35.0)	6 (16.7)		6 (40.0)	5 (20.8)	
DD	9 (45.0)	27 (75.0)		3 (20.0)	12 (50.0)	
Allele frequency			0.014[a]*			0.079[a]
I	15 (37.5)	12 (16.7)		18 (23.1)	19 (24.4)	
D	25 (62.5)	60 (83.3)		12 (15.4)	29 (37.2)	

p values calculated by [a] chi-square test and [b] Fisher's exact test. * Values are significant.

Table 3. Angiotensin-converting enzyme (ACE) allele and genotype frequencies in 60 patients with RA as per disease variables.

	Genotype				Allele Frequency		
	DD, n = 39 n (%)	ID, n = 11 n (%)	II, n = 10 n (%)	p	D, n = 89 n (%)	I, n = 31 n (%)	p
Sex				0.097[b]			0.061[a]
Male	11 (28.2)	2 (18.2)	6 (60.0)		24 (27.0)	14 (45.2)	
Female	28 (71.8)	9 (81.8)	4 (40.0)		65 (73.0)	17 (54.8)	
Nationality				0.076[b]			0.005[a]*
Arab	34 (87.2)	11 (100)	8 (80.0)		60 (67.4)	12 (38.7)	
Non-Arab	5 (12.8)	0 (0.0)	2 (20.0)		29 (32.6)	19 (61.3)	
Age at onset, yrs				0.694[b]			0.390[a]
≤ 40	24 (61.5)	7 (63.6)	6 (60.0)		38 (42.7)	16 (51.6)	
> 40	15 (38.5)	4 (36.4)	4 (40.0)		51 (57.3)	15 (48.4)	
Functional class				0.035[b]*			0.285[a]
Daily activity, work, recreation	13 (34.2)	7 (63.6)	5 (50.0)		33 (37.9)	17 (54.8)	
Daily activity, work	17 (44.7)	0 (0.0)	4 (40.0)		34 (39.1)	8 (25.8)	
Daily activity	8 (21.1)	4 (36.4)	1 (10.0)		20 (23.0)	6 (19.4)	

p values generated by [a] chi-square test, and [b] Fisher's exact test. * Values are significant.

risk of RA according to ACE genotype, using logistic regression. Step 1 included patients with RA and controls as the dependent variables, with genotype as per sex and nationality being independent variables. A person with genotype DD was found to be 3.25 times more at risk to develop RA than a person with II genotype (unadjusted OR 3.25, 95% CI 1.09–9.66, p = 0.034). ID genotype gave no significant risk. In step 2, when confounded by the sex variable, the risk conferred by DD genotype was reduced to 3.01. In step 3, when confounded by nationality, risk of DD increased significantly to 3.55. In step 4, when confounded by both sex and ethnicity, the relative risk for RA conferred by the DD genotype was 3.21 times that of the II genotype (95% CI 0.98–10.55, p = 0.055).

DISCUSSION

The genetic basis for rheumatologic diseases continues to be a fruitful area of research, with its potential implications in diagnosis, treatment, and disease outcome. The contribution of human leukocyte antigen (HLA) DR genes, particularly the HLA-DRB1 gene, to genetic predisposition of RA was the first described, and remains as the best characterized genetic risk factor contributing to RA[21]. The associations between RA and HLA-DR genes are, however, incomplete. It has recently been shown that 2 regions in the MHC, class II (DR-ß1) and class III (D6S273, HSP70, Bat2, TNFa), more completely define the risk for development of RA[22]. The above notwithstanding, it has been estimated that only 30% of the genetic contribution to RA can be attributed to HLA genes and it is suggested that other non-HLA genes play a role in RA susceptibility[1].

To view the genetic susceptibility to RA from another standpoint, patients with RA are believed to share some common pathogenic mechanisms that involve inflammation and elaboration of reactive oxygen species[2,3].

Recently, ACE, a key regulator in inflammatory signal transduction pathways, has been reported to be involved in pathogenesis of RA. Elevated synovial fluid ACE levels have been demonstrated, indicating that locally generated ACE could contribute to joint destruction in RA[4,5]. High levels of

Table 4. Risk of ACE gene polymorphism on RA estimated by logistic regression analysis.

Variable	Odds Ratio	95% CI	p
Step 1			
ACE			
II (reference)	1.00		
ID	0.85	0.26–2.78	0.783
DD	3.25	1.09–9.66	0.034
Step 2			
Sex			
Male	0.84	0.31–2.24	0.727
Female (reference)	1.00		
ACE			
II (reference)	1.00		
ID	0.78	0.22–2.79	0.704
DD	3.01	0.94–9.69	0.065
Step 3			
Nationality			
Arab	0.80	0.32–2.04	0.643
Non-Arab (reference)	1.00		
ACE			
II (reference)	1.00		
ID	0.88	0.27–2.94	0.838
DD	3.53	1.12–11.10	0.031
Step 4			
Sex			
Male	0.73	0.25–2.13	0.567
Female (reference)	1.00		
Nationality			
Arab	0.72	0.26–1.98	0.519
Non-Arab (reference)	1.00		
ACE			
II (reference)	1.00		
ID	0.78	0.22–2.80	0.707
DD	3.21	0.98–10.55	0.055

Step 1: unadjusted; Step 2: adjusted for sex; Step 3: adjusted for nationality; Step 4: adjusted for sex and nationality.

ACE have also been reported in RA pleural effusions[6]. The ACE gene, located on chromosome 17, plays a key role in the renin-angiotensin and kallikrein-kinin systems. ACE converts angiotensin I to angiotensin II (AT II) by release of the terminal His-Leu, resulting in increased vasoconstrictor activity, and also causes inactivation of bradykinin, a potent vasodilator. It is being increasingly realized that, beyond its hemodynamic effects, AT II has a role as a growth factor and as a proinflammatory modulator, participating in the key events of the inflammatory response[23,24].

The ACE gene locus is the major locus that determines serum ACE concentration. An I/D polymorphism of a 287 base-pair fragment in intron 16 of the human *ACE* gene has been found to account for 47% of the total variance of plasma ACE activity[7]. Plasma ACE activity is increased by 20%–40% for ID and 50%–70% for DD versus II genotypes ($p < 0.001$)[25]. It has been confirmed that serum ACE levels correspond to the order II < ID < DD in healthy individuals[8,9].

Since plasma and tissue levels of ACE are determined at the transcriptional level, we decided to investigate the hypothesis that the genotype of ACE in patients with RA may contribute to the onset and pathogenesis of this inflammatory disease. In our study, we detected a significant overrepresentation of the DD genotype and the D allele in patients with RA when compared to controls. Additionally, we found that sex correlates significantly with genotypic and allele frequencies, with male RA patients exhibiting a higher frequency of the DD genotype and D allele compared to male controls. Further, Arab patients show a higher frequency of D allele compared to Arab controls. No such differences exist in females and in non-Arabs. By logistic regression analysis, we found that the DD genotype confers a relative risk for development of RA of around 3. Our results also suggest a possible influence of the ACE gene on RA disease severity and functional classes.

ACE gene I/D polymorphisms are also implicated in the pathogenesis of a variety of cardiovascular disorders including myocardial infarction, left ventricular hypertrophy, and hypertension, and the ACE gene has been recognized as a top candidate gene for cardiovascular research[26]. A large number of studies have shown a positive association between the DD genotype and an increased risk of myocardial infarction[27]. A higher incidence of hypertension *per se*[28] and of left ventricular hypertrophy in patients with hypertension[29] has also been shown with this genotype, and also an increased risk of coronary heart disease and cardiovascular mortality in smokers[30]. Indeed it has been reported that the D allele behaves as a marker of atherosclerotic cardiovascular complications[31]. Thus, taken together, the available evidence supports the notion that the DD genotype adversely influences specific cardiovascular diseases. Given the association between RA and cardiovascular diseases[32-34], is it possible that the same pathway (ACE pathway) and the same genotype (DD) are involved in both these seemingly diverse conditions? This could enable this polymorphism and its association with RA, on the one hand, and cardiovascular diseases, on the other, to be placed in a broader perspective.

A significant association of the I allele has been demonstrated with the 3 clinical subclasses of juvenile idiopathic arthritis (JIA), with the highest association being observed in systemic JIA[35]. Thus, while the I allele predisposes to development of juvenile arthritis, our data suggest that the D allele increases the propensity to develop adult RA. These contrasting findings may be explained if one considers that these 2 disorders follow somewhat different pathways at the cellular and molecular level. Thus, a variety of recent data suggest that systemic-onset JIA, and possibly other JIA subtypes, are "autoinflammatory" diseases[36,37] in contrast to adult seropositive RA, which is an "autoimmune-inflammatory disease." It is therefore plausible that these 2 diseases follow diverse inflammatory pathways.

Our results suggest that the ACE gene contributes to the heritability of RA susceptibility. Our study is limited in its power, but being the first in this field, could provide impetus for larger and more powerful studies in other populations/eth-

nic groups to explore the relationship between ACE gene polymorphisms and susceptibility to RA.

ACKNOWLEDGMENT

The authors gratefully acknowledge the contribution of Joseph Edison Gomez, Chief Technician, Department of Community Medicine, Faculty of Medicine, Kuwait University, to statistical analysis of data.

REFERENCES

1. Orozco G, Rueda B, Martin J. Genetic basis of rheumatoid arthritis. Biomed Pharmacother 2006;60:656-62.
2. Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95.
3. McCulloch CA, Downey GP, El Gabalawy H. Signalling platforms that modulate the inflammatory response: new targets for drug development. Nat Rev Drug Discov 2006;5:864-76.
4. Cobankara V, Ozturk MA, Kiraz S, et al. Renin and angiotensin-converting enzyme (ACE) as active components of the local synovial renin-angiotensin system in rheumatoid arthritis. Rheumatol Int 2005;25:285-91.
5. Walsh DA, Catravas J, Wharton J. Angiotensin converting enzyme in human synovium: increased stromal [(125)I]351A binding in rheumatoid arthritis. Ann Rheum Dis 2000;59:125-31.
6. Soderblom T, Nyberg P, Pettersson T, Klockars M, Riska H. Pleural fluid beta-2-microglobulin and angiotensin-converting enzyme concentrations in rheumatoid arthritis and tuberculosis. Respiration 1996;63:272-6.
7. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86:1343-6.
8. Pullmann R Jr, Lukac J, Skerenova M, et al. Association between systemic lupus erythematosus and insertion/deletion polymorphism of the angiotensin converting enzyme (ACE) gene. Clin Exp Rheumatol 1999;17:593-6.
9. Papadopoulos KI, Melander O, Orho-Melander M, Groop LC, Carlsson M, Hallengren B. Angiotensin converting enzyme (ACE) gene polymorphism in sarcoidosis in relation to associated autoimmune diseases. J Intern Med 2000;247:71-7.
10. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
11. Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44-8.
12. Fransen J, Creemers MC, van Riel PL. Remission in rheumatoid arthritis: agreement of the Disease Activity Score (DAS28) with the ARA preliminary remission criteria. Rheumatology Oxford 2004;43:1252-5.
13. Pinals RS, Masi AT, Larsen RA. Preliminary criteria for clinical remission in rheumatoid arthritis. Arthritis Rheum 1981;24:1308-15.
14. Conaghan PG, Green MJ, Emery P. Established rheumatoid arthritis. Baillieres Best Pract Res Clin Rheumatol 1999;13:561-75.
15. Harris ED Jr. Treatment of active rheumatoid arthritis. UpToDate 2001 version 9.1 . 2001. Internet. Available at: www.utdol.com/utd/content/topic.
16. Scott DL, Houssien DA. Joint assessment in rheumatoid arthritis. Br J Rheumatol 1996;35 Suppl:14-8.
17. van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996;39:34-40.
18. Guidelines for the management of rheumatoid arthritis: 2002 Update. American College of Rheumatology Subcommittee on Rheumatoid Arthritis Guidelines. Arthritis Rheum 2002;46:328-46.
19. Sambrook J, Freitsch EF, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory; 1989.
20. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucl Acids Res 1992;20:1433.
21. Klareskog L, Padyukov L, Lorentzen J, Alfredsson L. Mechanisms of disease: Genetic susceptibility and environmental triggers in the development of rheumatoid arthritis. Nat Clin Pract Rheumatol 2006;2:425-33.
22. Singal DP, Li J, Zhu Y. Genetic basis for rheumatoid arthritis. Arch Immunol Ther Exp (Warsz) 1999;47:307-11.
23. Nataraj C, Oliverio MI, Mannon RB, et al. Angiotensin II regulates cellular immune responses through a calcineurin-dependent pathway. J Clin Invest 1999;104:1693-701.
24. Suzuki Y, Ruiz-Ortega M, Egido J. Angiotensin II: a double-edged sword in inflammation. J Nephrol 2000;13 Suppl:S101-S110.
25. Ishigami T, Iwamoto T, Tamura K, et al. Angiotensin I converting enzyme (ACE) gene polymorphism and essential hypertension in Japan. Ethnic difference of ACE genotype. Am J Hypertens 1995;8:95-7.
26. Mayer B, Schunkert H. ACE gene polymorphism and cardiovascular diseases [German]. Herz 2000;25:1-6.
27. Niu T, Chen X, Xu X. Angiotensin converting enzyme gene insertion/deletion polymorphism and cardiovascular disease: therapeutic implications. Drugs 2002;62:977-93.
28. Di Pasquale P, Cannizzaro S, Scalzo S, et al. Cardiovascular effects of I/D angiotensin-converting enzyme gene polymorphism in healthy subjects. Findings after follow-up of six years. Acta Cardiol 2005;60:427-35.
29. Celentano A, Mancini FP, Crivaro M, et al. Cardiovascular risk factors, angiotensin-converting enzyme gene I/D polymorphism, and left ventricular mass in systemic hypertension. Am J Cardiol 1999;83:1196-200.
30. Sayed-Tabatabaei FA, Schut AF, Vasquez AA, et al. Angiotensin converting enzyme gene polymorphism and cardiovascular morbidity and mortality: the Rotterdam Study. J Med Genet 2005;42:26-30.
31. Staessen JA, Wang JG, Ginocchio G, et al. The deletion/insertion polymorphism of the angiotensin converting enzyme gene and cardiovascular-renal risk. J Hypertens 1997;15:1579-92.
32. Chung CP, Avalos I, Raggi P, Stein CM. Atherosclerosis and inflammation: insights from rheumatoid arthritis. Clin Rheumatol 2007;26:1228-33. Epub 2007 Feb 2.
33. Chung CP, Oeser A, Solus JF, et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. Atherosclerosis 2007 Jan 29; [Epub ahead of print].
34. Del Rincon I, O'Leary DH, Freeman GL, Escalante A. Acceleration of atherosclerosis during the course of rheumatoid arthritis. Atherosclerosis 2006 Nov 9; [Epub ahead of print].
35. Alsaeid K, Haider MZ, Ayoub EM. Angiotensin converting enzyme gene insertion-deletion polymorphism is associated with juvenile rheumatoid arthritis. J Rheumatol 2003;30:2705-9.
36. Gattorno M, Martini A. Inherited autoinflammatory syndromes: an expanding new group of chronic inflammatory diseases. Clin Exp Rheumatol 2005;23:133-6.
37. Ravelli A, Martini A. Juvenile idiopathic arthritis. Lancet 2007;369:767-78.