Angiotensin converting enzyme gene polymorphism in Korean patients with primary knee osteoarthritis

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Abbreviations: ACE, angiotensin converting enzyme; OA, osteoarthritis; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism

Abstract

Angiotensin converting enzyme (ACE) plays an important role in the physiology of vasculature, blood pressure and inflammation. ACE gene, known to have insertion/deletion (I/D) polymorphism, has been widely investigated in its relation with cardiovascular and neurodegenerative diseases and longevity. ACE gene polymorphism in an inflammation associated osteoarthritis (OA) patients is not known. Here we have investigated ACE gene polymorphism in 142 Korean primary knee OA patients and 135 healthy volunteers to establish any clinical correlates between ACE polymorphism and knee osteoarthritis. Clinical parameters such as disease onset age, Kellgren-Lawrence grade and Lequesne's functional index provided additional analysis of the relationship of ACE polymorphism and clinical features of OA. Early onset OA showed significantly higher allele frequency and carriage rate of I than late onset OA. Radiographically severe and functionally poor OA showed higher carriage rate of I allele than radiogra-

phically mild and functionally good OA, respectively. This study first reports ACE gene polymorphism to be a risk factor for early onset, severe form primary knee OA.

Keywords: angiotensin converting enzyme; osteoarthritis; polymorphism; risk factor

Introduction

Angiotensin converting enzyme (ACE) is responsible for the conversion of angiotensin I to angiotensin II, which is a potent vasoconstrictor of the renin-angiotensin system, and it also inactivates bradykinin, a vasodilator of the kallikrein-kinin system (Viitanen *et al.*, 2001), which has major implication in inflammatory process including osteoarthritis (Cassim *et al.*, 2002). ACE gene insertion/deletion (I/D) polymorphism is localized in intron 16 of the human ACE gene and it corresponds to an *alu* repetitive sequence about 287 bp long (Asamoah *et al.*, 1996).

Osteoarthritis (OA) is the most common musculoskeletal disorder world-wide, and a multifactorial disorder in which aging, genetic, hormonal and mechanical factors are all major contributors to its onset and progression (Ghosh *et al.*, 2001). OA disorder suggested to be influenced by multiple genes as genetic risk factors in the form common population polymorphisms (Reginato *et al.*, 2002). Such genetic contribution for the development of OA was once estimated to be as high as 65% (MacGregor *et al.*, 1999; Shin *et al.*, 2001). In the patients with internal derangements and osteoarthritis of the temporomandibular joints, bradykinin and the score of synovitis shows positive correlation and higher detection rate of bradykinin among several pain-related mediators (Nishimura *et al.*, 2002). ACE is upregulated in synovial stroma in rheumatoid arthritis, thereby contributing to synovial hypoxia and proliferation (Walsh *et al.*, 2000; Min *et al.*, 2001). Therefore, ACE appears to show a great potential to influence the process of vascular control and joint inflammation.

In the present study, we explored the distribution of ACE genotype experimentally and the severity of knee OA clinically to investigate the correlation of ACE polymorphism with knee OA disease severity. This study suggests ACE gene polymorphism as a prognostic factor of OA.

Material and Methods

Study population

This study included 135 healthy control subjects and 160 patients with primary knee OA at the rheumatology clinic, Kyung Hee University Medical Center in Seoul, Korea. Inclusion criteria were primary OA with any symptom and/or sign of OA, positive finding in radiographs according to Kellgren-Lawrence grading, informed consent, and no evidence of arthritis due to other disease. This project has been reviewed and approved by the ethics review committee, the Medical Research Institute, Kyung Hee University Medical Center.

Healthy control subjects were chosen from adult volunteers cleared yearly health examinations. Primary knee OA patients were sequentially included in this study from March 1, 2002 to October 31, 2002 with rheumatologist's diagnosis and informed consent of patients. Total 135 control subjects and 142 patients constituted study population.

Clinical informations and classification of OA

Patient's history taking, physical examination and radiographic finding provided clinical informations of patients. In addition, demographic characteristics (age and sex), height, weight and date of onset, clinical features on disease severity were observed by way of Kellgren-Lawrence grade (Kellgren *et al.*, 1957) and Lequesne's functional index (Lequesne *et al.*, 1987). Kellgren-Lawrence grade represents disease severity reflected on radiographs and Lequesne's functional index represents functional or symptomatic status of patients.

The mean disease onset age of 52 years provided the classification of the early onset OA as the OA started before 52 year old and the late onset OA as the OA started above 52 years old. Radiographic findings of OA were classified into mild (Kellgren-Lawrence grade 1 or 2) or severe (Kellgren-Lawrence grade 3 or 4). Functional or symptomatic status of patients was classified into good (Lequesne's functional index ≤ 10) or poor (Lequesne's functional index > 10).

Genotype determination

Peripheral blood samples for DNA extraction from all subjects were collected in EDTA tube. Genomic DNA was extracted from the white blood cells with the use of DNA isolation kit for mammalian blood (MACHEREY-NAGEL GmbH & Co., Dren, Germany). The ACE gene I and D alleles were identified on the basis of polymerase chain reaction (PCR) amplification of the respective fragments from intron 16 of the gene, as previously described (Asamoah *et al.*, 1996). The

Figure 1. Electrophoresis of the PCR products of the ACE gene. Lane 1 is insertion homozygotes (II/II). Lane 2 and 3 are deletion homozygotes (D/D). Lane 4 is insertion/deletion type heterozygotes (I/D).

sense oligonucleotide primer used was 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', and the antisense primer was 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3' as previously published. 100 ng of template DNA was mixed with 10 pmole sense primer 0.5 μ l, 10 pmole antisense primer 0.5 μ l, 2.5 mM dNTP 0.7 μ l, *Taq* polymerase 0.3 μ l (BioTools, Canada), in a final volume of 40 μ l, and amplified through denaturation at 95°C for 2 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min, followed by final extension at 72°C for 5 min using Perkin Elmer GeneAmp PCR system 9600 (Roche Diagnostic Systems; Seo *et al.*, 2002). PCR products were analyzed with 2% agarose gel electrophoresis and ethidium bromide staining with one of following three patterns identified (Figure 1): a 490 bp fragment I (with insertion, genotype II), a 190 bp fragment D (without insertion, genotype DD) or both 490 and 190 bp fragments (with and without insertion, genotype ID).

Statistical analysis

The clinical data of subgroups with OA patients were compared using the unpaired t-test. The distribution of the ACE I/D genotypes for patients and control subjects were compared using the chi-square test. The odds ratios (OR) and 95% confidence intervals (CI) were calculated to quantify the association with early onset, severe form OA. All tests were 2-tailed, at the 5% level of significance. The SAS statistical package (release 6.12, SAS Institute Inc) was used.

Results

Subject characteristics

142 OA patients entered this study, 94 women, 48 men, mean $\pm$ SD age 58.6 ± 9.4 years (range 34-86), while 135 healthy control subjects consisted of 91 women and 44 men, mean $\pm$ SD age 59.9 ± 8.5 years (range 40-75) with no significant differences between

Table 1. Clinical characteristics of the 142 Korean primary knee osteoarthritis patients.

	All patients (n = 142)
Average age (year, mean $\pm$ SD)	58.6 $\pm$ 9.4
No. of female/male	94/48
Body mass index (kg/m ² , mean $\pm$ SD)	25.2 $\pm$ 3.0
Onset age (year, mean $\pm$ SD)	52.0 $\pm$ 9.3
Duration of OA (year, mean $\pm$ SD)	6.6 $\pm$ 6.7
Kellgren-Lawrence grade (No.)	
1	6
2	79
3	53
4	4
Lequesne's indices (mean $\pm$ SD)	10.1 $\pm$ 2.4

Table 2. Polymorphic distributions and allelic frequencies of ACE gene in osteoarthritis patients and Korean control subjects.

ACE gene	Patients n = 142 (%)	Controls n = 135 (%)	P value
Genotype			
II	51 (35.9)	44 (32.6)	NS
ID	68 (47.9)	58 (43.0)	
DD	23 (16.2)	33 (24.4)	
Allele			
I	59.9	54.1	NS
D	40.1	45.9	
Carrier			
I	83.8	75.6	NS
D	64.1	67.4	

NS, non-significant.

Table 3. Analysis of clinical subgroups by disease onset age.

	Early onset* n = 83 (%)	Late onset n = 59 (%)	OR (95%CI)	P value ^a	OR (95%CI)	P value ^b
Clinical subgroup						
K-L grade	2.3 $\pm$ 0.6	2.5 $\pm$ 0.7				
Functional index	10.1 $\pm$ 2.5	10.0 $\pm$ 2.3				
Genotype						
II	33 (39.8)	18 (30.5)		NS		NS
ID	43 (51.8)	25 (42.4)		NS		NS
DD	7 (8.4)	16 (27.1)	0.2 (0.1-0.6)	0.0029	0.3 (0.1-0.7)	0.0030
Allele						
I	66.2	51.9	1.8 (1.0-2.9)	0.0180	1.6 (1.1-2.4)	0.0171
D	33.8	48.1				
Carrier						
I	91.6	72.9	4.3 (1.8-9.9)	0.0004	3.6 (1.5-8.5)	0.0020
D	60.2	69.5		NS		NS

*The mean age at onset in the osteoarthritis (OA) patients was 52.0 year, so early onset is ≤ 52 year and late onset is > 52 year. Unpaired t-test of clinical subgroups was performed to compare early onset patients and late onset patients. But, statistical significance was not found.^aThe χ^2 test was performed to compare the genotype distribution, allele frequency and carriage rate of ACE gene in early onset OA patients with that in late onset OA patients. The P value was calculated at 2 degrees of freedom. ^bThe χ^2 test was performed to compare the ACE gene in early onset OA patients with that in normal controls. K-L grade, Kellgren-Lawrence grade; OR, odds ratio; CI, confidence interval; NS, non-significant.

two groups. Characteristics of patients such as body mass index, Kellgren-Lawrence grade, duration of OA, Lequesne's index are summarized in Table 1.

Distribution of ACE genotype in controls and patients

Distribution of genotype, allele frequencies and car-

riage rate in the control subjects and patients are shown in Table 2. For the ACE I/D polymorphism there was no significant difference in either the genotype distribution, the allele frequencies and carriage rate between control subjects and patients.

Table 4. Analysis of clinical subgroups by Kellgren-Lawrence grade.

	Severe* (Gr.3,4) <i>n</i> = 57 (%)	Mild (Gr.1,2) <i>n</i> = 85 (%)	OR (95%CI)	<i>P</i> value ^a	OR (95%CI)	<i>P</i> value ^b
Clinical subgroup						
Onset age	53.7 ± 9.1	51.0 ± 9.4				
Functional index	10.9 ± 2.4	9.4 ± 2.2				
Genotype						
II	21 (36.8)	30 (35.3)		NS		NS
ID	31 (54.4)	37 (43.5)		NS		NS
DD	5 (8.8)	18 (21.2)	0.4 (0.1-1.0)	0.0492	0.3 (0.1-0.8)	0.0128
Allele						
I	63.9	57.2		NS		NS
D	36.1	42.8				
Carrier						
I	91.2	78.8	2.7 (1.2-6.2)	0.0175	3.2 (1.4-7.3)	0.0043
D	63.2	64.7		NS		NS

*Grade 3 and 4 are severe progression, and Grade 1 and 2 are mild progression patients Kellgren-Lawrence grade. Unpaired t-test of clinical subgroups was performed to compare early onset patients and late onset patients. But, statistical significance was not found. ^aThe χ^2 test was performed to compare the genotype distribution, allele frequency and carriage rate of ACE gene in severe OA patients with that in mild OA patients. The *P* value was calculated at 2 degrees of freedom. ^bThe χ^2 test was performed to compare the ACE gene in severe OA patients with that in normal controls. OR, odds ratio; CI, confidence interval; NS, non-significant.

Effect of ACE polymorphism on the risk of early onset OA

Early onset OA patients are defined as those whose onset age ≤ 52 years, while late onset OA onset age > 52 years. The subgroups, clinical genotype, allele frequencies and carriage rates in the early onset OA group and late onset OA group are shown in Table 3. The frequency of II, ID, DD genotypes of ACE polymorphism were 33 (39.8%), 43 (51.8%), 7 (8.4%) in the early onset OA. The frequency of non-DD subtypes such as II and ID was significantly higher than that in late onset OA patients ($P = 0.0029$, OR = 0.2) or in normal controls ($P = 0.0030$, OR = 0.3).

Allelic frequencies and carriage rates showed significant difference between early onset and late onset groups ($P = 0.0180$, $P = 0.0004$ respectively), with the odd ratios of I allele 1.8 and 4.3 respectively. Allelic frequencies and carriage rates showed significant difference between early onset and normal control groups ($P = 0.0171$, $P = 0.0020$ respectively), with the odd ratios of I allele 1.6 and 3.6 respectively. Therefore, early onset OA patients showed higher allele frequency and carriage rate of I allele.

Effect of ACE polymorphism on the risk of radiographically severe OA

Radiographically severe OA patients are defined as those whose Kellgren-Lawrence grade 3 or 4, while

radiographically mild OA Kellgren-Lawrence grade 1 or 2. The genotype, allele frequencies and carriage rates between two groups are shown in Table 4. The frequency of II, ID, DD genotypes was 21 (36.8%), 31 (54.4%), 5 (8.8%) in the radiographically severe OA. The frequency of non-DD subtypes such as II and ID was significantly higher than that in mild OA patients ($P = 0.0492$, OR = 0.4) or in normal controls ($P = 0.0128$, OR = 0.3). Allelic frequencies showed no significant difference between severe OA and mild OA groups ($P > 0.05$), and between severe OA and normal control groups ($P > 0.05$) respectively. Carriage rates of I allele showed significant difference between severe OA and mild OA groups ($P = 0.0175$, OR = 2.7), and between severe OA and normal control groups ($P = 0.0043$, OR = 3.2) respectively. Therefore, radiographically severe OA patients showed higher carriage rate of I allele.

Effect of ACE polymorphism on the risk of functionally poor OA

Functionally or symptomatically poor OA patients are defined as those whose Lequesne's functional index > 10 , while functionally or symptomatically good OA patients are Lequesne's functional index ≤ 10 . The genotype, allele frequencies and carriage rates in the functionally poor OA patients group and functionally good OA patients group are shown in Table 5. The frequency of II, ID, DD genotypes was 21 (36.2%),

Table 5. Analysis of clinical subgroups by Lequesne's functional index.

	Poor index* n = 58 (%)	Good index n = 84 (%)	OR (95%CI)	P value ^a	OR (95%CI)	P value ^b
Clinical subgroup						
Onset age	53.1 ± 10.5	51.2 ± 8.5				
NK-L grade	2.5 ± 0.7	2.3 ± 0.5				
Genotype						
II	21 (36.2)	30 (35.7)		NS		NS
ID	33 (56.9)	35 (41.7)		NS		NS
DD	4 (6.9)	19 (22.6)	0.3 (0.1-0.8)	0.0124	0.2 (0.1-0.7)	0.0086
Allele						
I	64.7	56.5		NS		NS
D	35.3	43.5				
Carrier						
I	93.1	77.4	4.0 (1.6-9.7)	0.0015	4.2 (1.7-10.3)	0.0009
D	63.8	64.3		NS		NS

*Index score >10 is poor and score ≤10 is poor patients by Lequesne's functional index. Unpaired t-test of clinical subgroups was performed to compare early onset patients and late onset patients. But, statistical significance was not found. ^aThe χ^2 test was performed to compare the genotype distribution, allele frequency and carriage rate of ACE gene in poor OA patients with that in fair OA patients. The *P* value was calculated at 2 degrees of freedom. ^bThe χ^2 test was performed to compare the genotype distribution of ACE gene in poor OA patients with that in normal controls. K-L grade, Kellgren-Lawrence grade; OR, odds ratio; CI, confidence interval; NS, non-significant.

33 (56.9%), 4 (6.9%) in the functionally poor OA. The frequency of non-DD subtypes such as II and ID was significantly higher than that in functionally good OA patients ($P = 0.0124$, OR = 0.3) or in normal controls ($P = 0.0086$, OR = 0.2). Allelic frequencies showed no significant differences between poor OA and good OA groups ($P > 0.05$), and between severe OA and normal control groups, respectively ($P > 0.05$). Carriage rates of I allele showed significant difference between poor OA and good OA groups ($P = 0.0015$, OR = 4.0), and between poor OA and normal control groups ($P = 0.0009$, OR = 4.2) respectively.

Discussion

Primary OA is a common and multifactorial disease with major genetic component (s) and difficult to analyze due to its high frequency in the general population and extensive clinical heterogeneity (Loughlin *et al.*, 2002). Until now, there has not been any such study for a possible association between ACE gene polymorphism and primary OA, especially knee patients. In this study, we have examined any correlates between with the clinical severity of knee OA and ACE polymorphisms in a genetically homogeneous Korean population. The distributions of the D and I alleles and the DD, ID and II genotypes in control subjects were different from that in Caucasian po-

pulation. Generally, ethnic origin should be carefully considered while studying the association between genetic factors and disease susceptibility due to the presence of significant discrepancies of genetic polymorphism among populations of different genetic background. Allele frequencies of the ACE gene polymorphism have been reported to vary among racial and ethnic groups. For example, the frequency of the D allele in normal Caucasians is 50-58%, but 35-39% in normal Chinese (Jeng *et al.*, 1997; Sharma *et al.*, 1998; Lin *et al.*, 2002). In our study, the frequency of I > D allele and the distributions rate of ID > II > DD in the control subjects are similar to the previously reported distributions for Japanese (Ishigami *et al.*, 1995), Chinese (Lee *et al.*, 1994) and Koreans (Park *et al.*, 1997). Therefore, it is possible that an association study of the ACE gene and primary OA between Korean and Caucasian might have different results.

In humans, the levels of both plasma ACE and peripheral cellular ACE are genetically determined (Cambien *et al.*, 1988; Rigat *et al.*, 1990), but there are some who report no associated with genetic factor (McKenzie *et al.*, 1995). A deletion (D) polymorphism in the ACE gene has been found to be significantly associated with increased risk of myocardial infarction in some Caucasians (Cambien *et al.*, 1992), essential hypertension in African Americans (Asamoah *et al.*, 1996), higher left ventricular mass index

and thicker wall (Gharavi *et al.*, 1996), coronary heart disease, myocardial infarction and both diabetic and non-diabetic renal disease (Kennon *et al.*, 1999). On the other hand, I allele has been associated with the risk for Alzheimer's disease and the increase of human longevity (Schachter *et al.*, 1994; Kehoe *et al.*, 1999). However, the association between I/D polymorphism and disease susceptibility is controversial in the Parkinson's disease (Lin *et al.*, 2002) and autoimmune manifestations in sarcoidosis (Papadopoulos *et al.*, 2000).

In this study, there was no significant difference in the distribution of genotype or allelic frequency between control subjects and patients with primary OA. These results suggest that the ACE polymorphism is not involved in primary knee OA. In addition, analysis for the association of OA patient by clinical parameters and genotype distribution showed a positive association of I carriage (non-DD genotype) with early onset, functionally poor and radiographically severe primary knee OA. The allele frequency of I was significantly higher in early onset OA. This finding suggests that I allele has been associated with the risk for severity of primary OA disease, especially early onset OA. In OA patients, plasma and tissue kallikreins levels were elevated in synovial fluid, kinin levels raised and kinin B2 receptors in synovial lining cells up-regulated (Cassim *et al.*, 1997). Kinins have been implicated in the pathogenesis of experimental and clinical inflammatory arthritis. Therefore, we could speculate that the I allele of ACE gene polymorphism may be a link between the clinical features of OA and ACE activity, especially effects on kallikrein-kininogen system of ACE.

In conclusion, this finding of ACE gene polymorphism associated with the severe form primary knee OA patients could serve as a plausible risk factor of early onset. Further study on a larger population remains to be conducted for a definitive affirmation of correlates of ACE polymorphism on the arthritis.

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References

- Asamoah A, Yanamandra K, Thurmon TF, Richter R, Green R, Lakin T, Martin C. A deletion in the angiotensin converting enzyme (ACE) gene is common among African Americans with essential hypertension. *Clin Chim Acta* 1996;254:41-6
- Cambien F, Alhenc-Gelas F, Herbeth B, Andre JL, Rakotavao R, Gonzales, MF, Allegrini J, Bloch C. Familial resemblance of plasma angiotensin-converting enzyme level: The Nancy study. *Am J Hum Genet* 1988;43:774-80
- Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas P, Souvrieff F. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 1992;359:641-4
- Cassim B, Naidoo S, Ramsaroop R, Bhoola KD. Immunolocalization of bradykinin receptors on human synovial tissue. *Immunopharmacology* 1997;36:121-5
- Cassim B, Mody G, Bhoola KD. Kallikrein cascade and cytokines in inflamed joints. *Pharmacol Ther* 2002;94:1-34
- Gharavi AG, Lipkowitz MS, Diamond JA, Jhang JS, Phillips RA. Deletion polymorphism of the angiotensin-converting enzyme gene is independently associated with left ventricular mass and geometric remodeling in systemic hypertension. *Am J Cardiol* 1996;77:1315-9
- Ghosh P, Cheras PA. Vascular mechanisms in osteoarthritis. *Best Pract Res Clin Rheumatol* 2001;15:693-709
- Ishigami T, Iwamoto T, Tamura K, Yamaguchi S, Iwasawa K, Uchino K, Umemura S, Ishii M. Angiotensin I converting enzyme (ACE) gene polymorphism and essential hypertension in Japan. Ethnic difference of ACE genotype. *Am J Hypertens* 1995;8:95-7
- Jeng JR, Harn HJ, Jeng CY, Yueh KC, Shieh SM. Angiotensin I converting enzyme gene polymorphism in Chinese patients with hypertension. *Am J Hypertens* 1997;10:558-61
- Kehoe PG, Russ C, Mclory S, Williams H, Holmans P, Holmes C, Liolitsa D, Vahidassr D, Powell J, McGleenon B, Liddell M, Plomin R, Dynan K, Williams N, Neal J, Cairns NJ, Wilcock G, Passmore P, Lovestone S, Williams J, Owen MJ. Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease. *Nat Genet* 1999;21:71-2
- Kellgren JK, Lawrence JS. Radiological assessment of osteoarthritis. *Ann Rheum Dis* 1957;16:494-501
- Kennon B, Petrie JR, Small M, Connell JM. Angiotensin-converting enzyme gene and diabetes mellitus. *Diabet Med* 1999;16:448-58
- Lee EJ. Population genetics of the angiotensin-converting enzyme gene in Chinese. *Br J Clin Pharmacol* 1994;37:212-4
- Lequesne M, Mry C, Samson M, Gérard P. Indexes of severity for osteoarthritis of the hip and knee. Validation-value in comparison with other assessment tests. *Scand J Rheumatol* 1987;65:85-9
- Lin JJ, Yueh KC, Chang DC, Lin SZ. Association between genetic polymorphism of angiotensin-converting enzyme gene and Parkinson's disease. *J Neurol Sci* 2002;199:25-9
- Loughlin J, Dowling B, Mustafa Z, Chapman K. Association of the interleukin-1 gene cluster on chromosome 2q13 with knee osteoarthritis. *Arthritis Rheum* 2002;46:1519-27
- MacGregor AJ, Spector TD. Twins and the genetic architecture of osteoarthritis. *Rheumatology (Oxford)* 1999;38:583-8
- McKenzie CA, Julier C, Forrester T, McFarlane-Anderson N,

- Keavney B, Lathrop GM, Ratcliffe PJ, Farrall M. Segregation and linkage analysis of serum angiotensin I-converting enzyme levels: evidence for two quantitative-trait loci. *Am J Hum Genet* 1995;57:1426-35
- Min BH, Kim HJ, Lim H, Park CS, Park SR. Effects of ageing and arthritic disease on nitric oxide production by human articular chondrocytes. *Exp Mol Med* 2001;33:299-302
- Nishimura M, Segami N, Kaneyama K, Suzuki T, Miyamaru M. Relationships between pain-related mediators and both synovitis and joint pain in patients with internal derangements and osteoarthritis of the temporomandibular joint. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;94:328-32
- 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
- Park HY, Kwon HM, Kim D, Jang Y, Shim WH, Cho SY, Kim HS. The angiotensin converting enzyme genetic polymorphism in acute coronary syndrome-ACE polymorphism as a risk factor of acute coronary syndrome. *J Korean Med Sci* 1997;12:391-7
- Reginato AM, Olsen BR. The role of structural genes in the pathogenesis of osteoarthritic disorders. *Arthritis Res* 2002;4:337-45
- 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
- Schachter F, Faure-Delanef L, Guenot F, Rouger H, Froguel P, Lesueur-Ginot L, Cohen D. Genetic associations with human longevity at the APOE and ACE loci. *Nat Genet* 1994;6:29-32
- Seo JC, Han SW, Yin CS, Koh HK, Kim CH, Kim EH, Leem KH, Lee HS, Park HJ, Kim SA, Choe BK, Lee HJ, Yim SV, Kim CJ, Chung JH. Evaluation of a Apo-1/Fas promoter polymorphism in Korean stroke patients. *Exp Mol Med* 2002;34:294-8
- Sharma P. Meta-analysis of the ACE gene in ischemic stroke. *J Neurol Neurosurg Psychiatry* 1998;64:227-30
- Shin HD. Host genetic epidemiology by single nucleotide polymorphism (SNP) analysis. *Exp Mol Med* 2001;33:51-69
- Viitanen L, Pihlajami J, Halonen P, Lehtonen M, Kareinen A, Lehto S, Laakso M. Association of angiotensin converting enzyme and plasminogen activator inhibitor-1 promoter gene polymorphisms with features of the insulin resistance syndrome in patients with premature coronary heart disease. *Atherosclerosis* 2001;157:57-64
- 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