

Angiotensin-Converting Enzyme Insertion/Deletion Polymorphism and Systemic Lupus Erythematosus: A Metaanalysis

YOUNG HO LEE, YOUNG HEE RHO, SEONG JAE CHOI, JONG DAE JI, and GWAN GYU SONG

ABSTRACT. *Objective.* To explore whether insertion (I) and deletion (D) polymorphisms within intron 16 of the angiotensin-converting enzyme (ACE) gene confer susceptibility to systemic lupus erythematosus (SLE) and lupus nephritis (LN).

Methods. We surveyed studies of ACE I/D polymorphism and SLE using Medline and manual searches. We conducted a metaanalysis of the DD genotype (recessive effect), DD and DI genotype (dominant effect), and D allele of the ACE overall and in each ethnic population. We performed a metaanalysis of ACE I/D polymorphism in SLE and LN.

Results. Thirteen comparison studies were included in our metaanalysis consisting of 1411 patients with SLE and 1551 controls. We found no association of ACE I/D polymorphism with SLE in the total sample and by ethnic groups. There was a trend for association of the DD genotype (OR 1.212, 95% CI 0.966–1.520, $p = 0.097$) and the D allele with SLE in Caucasian patients (OR 1.157, 95% CI 0.991–1.349, $p = 0.064$); however, this was not statistically significant. The metaanalysis also showed no association of the ACE I/D polymorphisms with LN.

Conclusion. This metaanalysis of 2962 subjects showed there is a lack of association of the ACE I/D polymorphism with SLE and LN. (J Rheumatol 2006;33:698–702)

Key Indexing Terms:

SYSTEMIC LUPUS ERYTHEMATOSUS
ANGIOTENSIN-CONVERTING ENZYME

POLYMORPHISMS
METAANALYSIS

Systemic lupus erythematosus (SLE) is the prototype of human autoimmune diseases and is a disorder of generalized autoimmunity with unknown etiology, characterized by intense inflammation and multiple organ damage. Lupus nephritis (LN) remains a predominant cause of morbidity and mortality in SLE¹.

The angiotensin-converting enzyme (ACE) activates angiotensin I into angiotensin II and inactivates bradykinin via the kallikrein-kininogen system. Angiotensin II, the main effector molecule of the renin-angiotensin system, is a pleiotropic molecule and a mediator of the development and progression of renal disease². In addition, angiotensin II is a potent proinflammatory modulator that augments and perpetuates immune responses³. The ACE gene, located on chromosome 17q23, contains an insertion (I) and deletion (D) poly-

morphism within intron 16 consisting of the presence or absence of a 287 bp repeat sequence⁴. The DD genotype is associated with about 2-fold higher tissue and plasma concentrations of ACE than the II genotype⁴. Thus it seems to be possible that the D allele could play a role in the pathogenesis of SLE and LN. As a candidate gene approach, ACE I/D polymorphisms in SLE and LN have been studied^{5–14}. Some studies have shown increased D allele in SLE and/or LN^{11,12}, others have shown no association or inverse association^{5–10,13,14}. The role of the ACE polymorphism remains unclear in the pathogenesis of SLE and LN.

Individual studies with small sample sizes have insufficient statistical power to detect a positive association and more so to demonstrate an absence of association. To confirm a lack of association larger sample sizes are required. The low statistical power of individual studies to detect small differences between cases and controls may be a factor to explain the lack of conclusive results. Metaanalysis is a powerful method to overcome the problem of small sample size and inadequate statistical power of genetic studies of complex traits. Metaanalysis integrates previous research, providing increased statistical power and resolution by pooling the results of independent analyses¹⁵. We investigated whether the ACE I/D functional polymorphism contributes to the susceptibility of SLE and LN, using a metaanalysis of published data.

From the Division of Rheumatology, Department of Internal Medicine, Korea University Medical Center, College of Medicine, Korea University, Seoul, Korea.

Y.H. Lee, MD, PhD, Assistant Professor of Rheumatology; Y.H. Rho, MD, Clinical Instructor of Rheumatology; S.J. Choi, MD, Clinical Instructor of Rheumatology; J.D. Ji, MD, PhD, Associate Professor of Rheumatology; G.G. Song, MD, PhD, Professor of Rheumatology.

Address reprint requests to Dr. Y.H. Lee, Division of Rheumatology, Department of Internal Medicine, Korea University Medical Center, 126-1 Ka, Anam-Dong, Seongbuk-Ku, Seoul, 136-705, Korea.
E-mail: lyhcg@korea.ac.kr

Accepted for publication October 26, 2005.

MATERIALS AND METHODS

Identification of eligible studies and data extraction. We performed an exhaustive search on studies that examined the association of *ACE* I/D polymorphisms with SLE. A literature search was carried out using Medline citation to identify available articles in which the *ACE* I/D polymorphism was determined in SLE patients and controls (the most recent report was February 2005). All references in the studies were reviewed to identify additional works not indexed by Medline. The following key words and subject terms were searched: “angiotensin-converting enzyme,” “*ACE*,” “systemic lupus erythematosus,” and “SLE.” We extracted the available genotype and allele frequencies of the *ACE* polymorphism from each study.

Evaluation of publication bias. A funnel plot is used to detect publication bias. However, due to the limitations of a funnel plot, which needs a range of studies with varying size and subjective judgment, we have evaluated publication bias using Egger’s linear regression test¹⁶. Egger’s test measures funnel plot asymmetry on the natural logarithm scale of the odds ratio (OR).

Evaluation of statistical association. Allele frequencies at the *ACE* polymorphism from the respective studies were determined by the allele counting method. A chi-square test was used to determine if observed frequencies of genotypes conformed to Hardy-Weinberg expectations.

The point estimates of the risk, the OR, and 95% confidence interval (CI) were estimated for each study. We assessed the within- and between-study variation or heterogeneity by testing Cochran’s Q statistic¹⁷. The heterogeneity test assessed the null hypothesis that all studies were evaluating the same effect. If the significant Q statistic ($p < 0.10$) indicated heterogeneity across studies, the random effect model was used for metaanalysis. Otherwise a fixed effect model was used. The fixed effect model assumes all studies are estimating the same underlying effect and considers only within-study variation. We also quantified the effect of heterogeneity by using a recently developed measure, $I^2 = 100\% \times (Q - df)/Q$ ¹⁸. The I^2 measures the degree of inconsistency in studies by calculating the percentage of total variation across studies that is due to heterogeneity rather than by chance. Finally, the overall or pooled estimate of risk was obtained using Peto’s method in the fixed effect model and by the method of DerSimonian and Laird in the random effect model^{19,20}. Pooled OR in the metaanalysis is performed weighting individual OR by the inverse of their variance. Statistical manipulations of metaanalysis were undertaken using the EasyMA program (available from: <http://www.spc.univ-lyon1.fr/easyma.dos/>).

RESULTS

Studies included in the metaanalysis. Twelve relevant studies

concerning the *ACE* I/D polymorphism and SLE were identified through Medline^{21,22}. Two studies were excluded due to no case-control study data²¹ and data duplication²². Three of the eligible studies contained subjects from 2 different ethnic groups^{6,9,14}. Therefore, a total of 13 separate comparisons were considered, consisting of 1411 patients with SLE and 1551 controls. These 13 studies comprise 6 Caucasian, 3 African, and 4 Asian populations. The features of studies in the metaanalysis are given in Table 1.

Heterogeneity and publication bias. There was no between-study heterogeneity in the analysis of the DD genotype and D allele in Caucasians, and the DD and DI genotype in Africans; these metaanalyses were performed following a fixed effect model, and the other metaanalyses were done in a random effect model (Table 2). Publication bias, a bias toward publishing positive results, would result in the inclusion of a disproportionate number of positive studies, misrepresenting the actual findings. Publication bias can be a problem in performing metaanalysis. However, there was no evidence of publication bias in this metaanalysis (Egger’s regression test p values > 0.1).

Metaanalysis of the ACE I/D polymorphism and SLE. The *ACE* I/D polymorphisms in the control populations, overall and by ethnic group, were in Hardy-Weinberg proportion. We performed metaanalysis of the DD genotype (recessive effect), DD and DI genotype (dominant effect), and D allele of the *ACE* in the total sample and by ethnic groups. The summary for the *ACE* polymorphism in SLE is shown in Table 2. Metaanalysis of the DD genotype, DD and DI genotype, and D allele revealed no association with SLE in the overall group and each ethnic population. There was a trend to association of the DD genotype and D allele with SLE in Caucasians; however, this was not statistically significant. The overall OR of the DD genotype and the D allele in Caucasians were 1.212 (95% CI 0.996–1.520, $p = 0.097$) and 1.157 (95% CI 0.991–1.349, $p = 0.064$), respectively (Figure 1).

Table 1. Characteristics of individual studies included in metaanalysis.

Study	Ethnicity	SLE, N	Control, N	Mean Age, yrs		Male/Female		D Alleles, %		OR	95% CI
				SLE	Control	SLE	Control	SLE	Control		
Saeed ⁵	Asian	39	79	33	35	NA	NA	46.2	41.8	1.195	0.692–2.063
Douglas ⁶	African	140	70	39	40	NA	NA	58.6	58.6	1.000	0.662–1.510
Douglas ⁶	Caucasian	85	201	39	40	NA	NA	58.8	55.5	1.147	0.797–1.649
Uhm ⁷	Asian	211	114	35	27	21/190	NA	40.5	40.8	0.989	0.712–1.373
Prkacin ⁸	Caucasian	18	21	38	49	2/16	3/18	63.9	50.0	1.769	0.712–4.397
Kaufman ⁹	African	128	129	NA	NA	NA	NA	66.8	59.7	1.359	0.984–1.947
Kaufman ⁹	Caucasian	206	291	NA	NA	NA	NA	51.7	54.0	0.914	0.709–1.176
Molad ¹⁰	Caucasian	56	48	41	41	5/51	5/51	76.8	69.8	1.432	0.771–2.657
Pullmann ¹¹	Caucasian	101	148	43	47	3/98	47/101	62.9	52.0	1.561	1.083–2.250
Guan ¹²	Asian	144	150	32	NA	16/128	54/96	45.1	25.7	2.383	1.683–3.374
Akai ¹³	Asian	84	100	40	33	10/74	1/99	30.4	40.0	0.654	0.424–1.009
Tassiulas ¹⁴	Caucasian	121	122	NA	NA	NA	NA	67.4	62.7	1.227	0.845–1.783
Tassiulas ¹⁴	African	78	78	NA	NA	NA	NA	59.0	72.4	0.547	0.340–0.879
Total		1411	1551					54.1	51.5	1.145	0.912–1.428

NA: not available.

Table 2. Metaanalysis of *ACE* I/D polymorphisms and association with SLE.

Polymorphism	Population	Sample Size		No. of Studies	Test of Association			Model	Test of heterogeneity		I ²
		SLE	Control		OR	95% CI	p		Q	p	
DD genotype	Overall	1411	1551	13	1.300	0.941–1.795	0.11	R	43.5	< 0.001	72
	Caucasian	587	831	6	1.212	0.966–1.520	0.097	F	3.75	0.59	0
	African	346	277	3	0.875	0.353–2.167	0.77	R	14.7	< 0.001	80
	Asian	478	443	4	1.820	0.733–4.517	0.38	R	16.8	< 0.001	82
DD + DI genotype	Overall	1411	1511	13	1.094	0.844–1.417	0.50	R	22.2	0.035	46
	Caucasian	587	831	6	1.374	0.870–2.166	0.19	R	9.84	0.08	39
	African	346	277	3	0.963	0.622–1.491	0.87	F	0.24	0.89	0
	Asian	478	443	4	0.941	0.549–1.613	0.46	R	11.0	0.012	73
D allele	Overall	2822	3102	13	1.145	0.912–1.428	0.21	R	42.5	< 0.001	72
	Caucasian	1174	1662	6	1.157	0.991–1.349	0.064	F	7.29	0.20	18
	African	692	554	3	0.923	0.557–1.531	0.76	R	8.97	0.011	67
	Asian	956	886	4	1.172	0.666–2.063	0.58	R	23.9	<0.001	87

F: fixed effect model; R: random effect model.

Metaanalysis of association between ACE polymorphism and LN. Three studies were included in the metaanalysis for the association of *ACE* I/D polymorphism with LN^{9,12,13}. Metaanalysis of the DD genotype, DD and DI genotype, and D allele showed no association with LN. The overall OR of the association of the D allele with LN was 1.293 (95% CI 0.564–2.964, $p = 0.54$; Table 2).

DISCUSSION

ACE is expressed in a wide range of tissues such as kidney, heart, lung, vascular endothelium, and testes. *ACE* plays an important role in the renin-angiotensin system: angiotensin II, converted by *ACE*, increases vascular smooth muscle cell contraction and affects smooth muscle proliferation, monocyte adhesion, and platelet adhesion and aggregation. Angiotensin II also is a potent proinflammatory modulator³. The *ACE* I/D polymorphism is located in an intron of the *ACE* gene, and this polymorphism is in strong linkage disequilibrium with genetic factors that influence serum *ACE* concentrations⁴. It is not believed to have a direct effect on *ACE* expression. The *ACE* I/D polymorphism accounts for about one-half of the variance in *ACE* plasma levels in humans. The *ACE* I/D polymorphism has been studied in several diseases including Alzheimer disease, myocardial infarction, cerebral infarct, hypertension, and diabetic nephropathy^{23–27}. These studies have shown an association of the DD genotype and D allele in these diseases.

Based on the biological function³ and chromosomal location⁴, *ACE* has been considered one of the possible candidate genes in SLE, where vasculitis and vascular changes are frequently found. Studies on *ACE* I/D polymorphism in SLE have shown controversial results^{5–14}. This discrepancy is not surprising; there are several possible explanations for the controversial results, such as clinical heterogeneity, ethnic difference or real genetic heterogeneity, small sample sizes, and inadequate statistical power, but the exact reason remains unclear. Metaanalysis is a useful tool for summarizing incon-

sistent results from different studies by increasing the sample size and statistical power. Thus, these inconsistent results about the *ACE* I/D polymorphism in SLE and LN led us to do this metaanalysis of the published data to clarify the role of the *ACE* I/D polymorphism in SLE and LN. In our metaanalysis, there was no association of the DD genotype, DD and DI genotype, and D allele and SLE in the overall population. Allele frequency for the *ACE* I/D polymorphism may differ considerably between ethnic groups. To rule out an effect of ethnic heterogeneity, we compared the *ACE* I/D polymorphism in each ethnic group, such as Caucasian, African, and Asian. Although there was a trend to association of the DD genotype and D allele with SLE in Caucasian patients, it was not statistically significant; and there was not an association of the *ACE* I/D polymorphism with LN.

We could find no evidence of an association of the *ACE* I/D polymorphism with SLE and LN in this metaanalysis. Our data are consistent with the results of one large family-based association study of 644 SLE families that showed no significant association of the *ACE* I/D polymorphism in all families and Caucasians²². The family-based study showed that the *ACE* I/D polymorphism was associated with SLE among non-Caucasians (61% transmission; $p = 0.026$), but the ethnicity of the non-Caucasians was not clearly defined. We also did metaanalysis of the II genotype and I allele in SLE and LN, but we found no association similar to the results from the DD genotype and D allele (data not shown).

There were some limitations in our study. First, the number of studies included in the metaanalysis might be not enough to detect the association with small effect in ethnic groups and LN group. Three studies were contained in the metaanalysis of LN where one study indicated the positive association of the D allele with LN¹², and others did not^{9,13}. Second, interpretation of this metaanalysis can not be ascertained due to association of the *ACE* I/D polymorphism with various common phenotypes such as hypertension, chronic renal failure, Alzheimer's disease or thromboembolism. We could not know

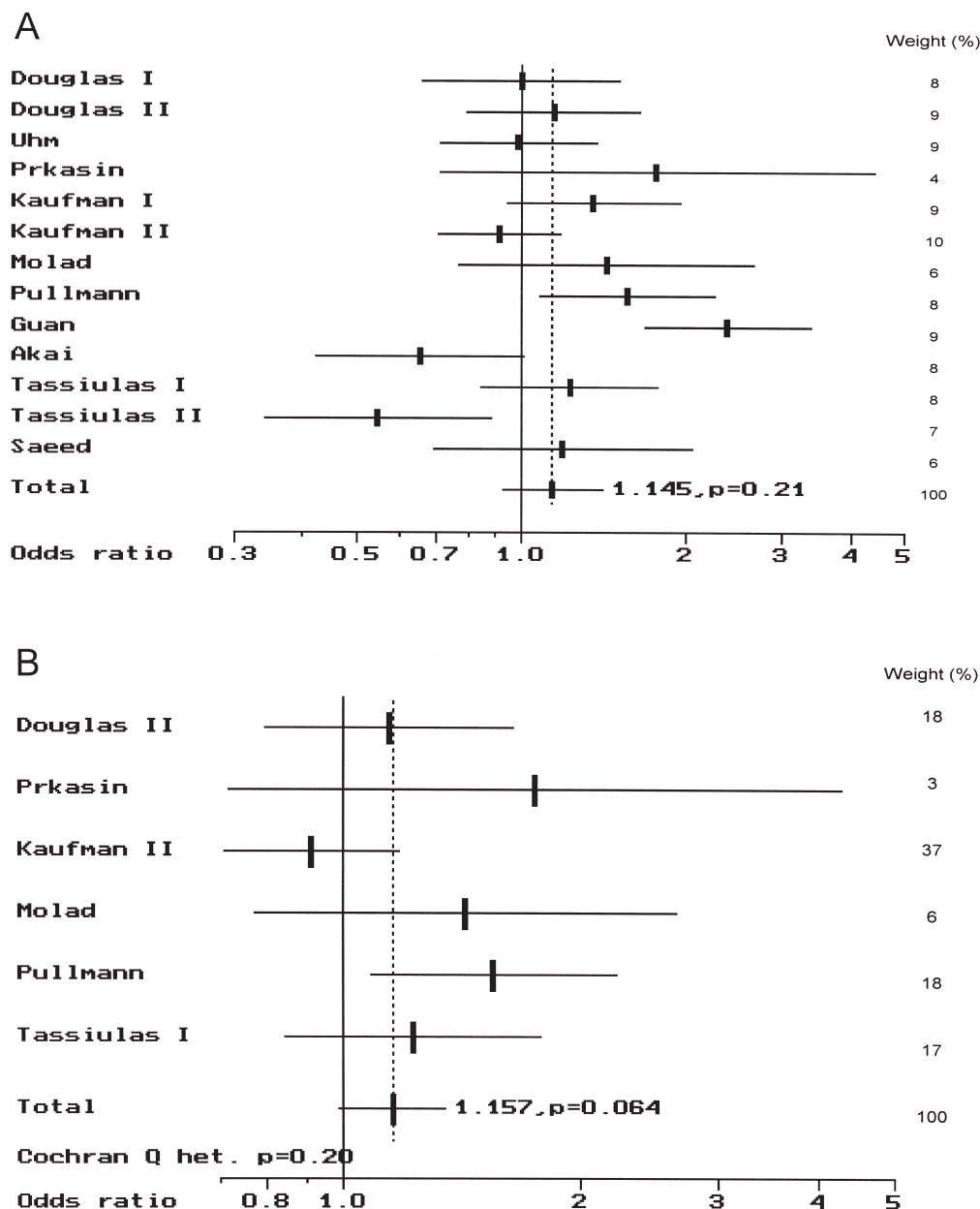


Figure 1. OR and 95% CI of individual studies and pooled data for the association of the D allele of the *ACE* I/D polymorphism and SLE in the total (A) and in the Caucasian samples (B).

whether the *ACE* I/D polymorphism is associated with chronic renal failure due to the difference between LN and chronic renal failure that may be independently associated with the *ACE* I/D polymorphisms. Third, the I/D polymorphism was not the only *ACE* polymorphism that has been studied in different lupus populations. Other *ACE* polymorphisms such as *ACE* G2350A have been studied in SLE, but we could not do metaanalysis of other *ACE* polymorphisms because of a few studies. Fourth, we could not adjust the results by age and gender because the data were not available for the adjustment by age and gender. Fifth, it is interesting to examine whether

the *ACE* I/D polymorphism is associated with the lupus activity or clinical features, but we could not examine this due to very few data.

In conclusion, this metaanalysis with the published data demonstrates no association of the *ACE* I/D polymorphism with SLE and LN. These data do not support that the *ACE* I/D polymorphisms play an important role in SLE and LN.

REFERENCES

1. Boumpas DT, Fessler BJ, Austin HA III, Balow JE, Klippel JH, Lockshin MD. Systemic lupus erythematosus: emerging concepts.

- Part 2: Dermatologic and joint disease, the antiphospholipid antibody syndrome, pregnancy and hormonal therapy, morbidity and mortality, and pathogenesis. *Ann Intern Med* 1995;123:42-53.
2. Egidio J. Vasoactive hormones and renal sclerosis. *Kidney Int* 1996;49:578-97.
 3. Weinstock JV, Blum AM, Kassab JT. Angiotensin II is chemotactic for a T-cell subset which can express migration inhibition factor activity in murine schistosomiasis mansoni. *Cell Immunol* 1987;107:180-7.
 4. 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.
 5. Saeed M, Mekan SF, Rabbani MA, Arain FM, Arif M, Shaharyar S. Angiotensin converting enzyme (ACE) gene polymorphisms and lupus disease severity: a promising link. *Ann Rheum Dis* 2005;64:164-5.
 6. Douglas G, Reilly C, Dooley MA, Page G, Cooper G, Gilkeson G. Angiotensin-converting enzyme (insertion/deletion) and endothelial nitric oxide synthase polymorphisms in patients with systemic lupus erythematosus. *J Rheumatol* 2004;31:1756-62.
 7. Uhm WS, Lee HS, Chung YH, et al. Angiotensin-converting enzyme gene polymorphism and vascular manifestations in Korean patients with SLE. *Lupus* 2002;11:227-33.
 8. Prkacin I, Novak B, Sertic J, Mrzljak A. Angiotensin-converting enzyme gene polymorphism in patients with systemic lupus. *Acta Med Croatica* 2001;55:73-6.
 9. Kaufman KM, Kelly J, Gray-McGuire C, et al. Linkage analysis of angiotensin-converting enzyme (ACE) insertion/deletion polymorphism and systemic lupus erythematosus. *Mol Cell Endocrinol* 2001;177:81-5.
 10. Molad Y, Gal E, Magal N, et al. Renal outcome and vascular morbidity in systemic lupus erythematosus (SLE): lack of association with the angiotensin-converting enzyme gene polymorphism. *Semin Arthritis Rheum* 2000;30:132-7.
 11. 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.
 12. Guan T, Liu Z, Chen Z. Angiotensin-converting enzyme gene polymorphism and the clinical pathological features and progression in lupus nephritis. *Zhonghua Nei Ke Za Zhi* 1997;36:461-4.
 13. Akai Y, Sato H, Iwano M, et al. Association of an insertion polymorphism of angiotensin-converting enzyme gene with the activity of lupus nephritis. *Clin Nephrol* 1999;51:141-6.
 14. Tassioulas IO, Aksentijevich I, Salmon JE, Kim Y, et al. Angiotensin I converting enzyme gene polymorphisms in systemic lupus erythematosus: decreased prevalence of DD genotype in African American patients. *Clin Nephrol* 1998;50:8-13.
 15. Egger M, Smith GD, Phillips AN. Meta-analysis: principles and procedures. *BMJ* 1997;315:1533-7.
 16. Egger M, Davey SG, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629-34.
 17. Davey Smith G, Egger M. Meta-analyses of randomised controlled trials [letter]. *Lancet* 1997;350:1182.
 18. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;21:1539-58.
 19. Yusuf S, Peto R, Lewis J, Collins R, Sleight P. Beta blockade during and after myocardial infarction: an overview of the randomized trials. *Prog Cardiovasc Dis* 1985;27:335-71.
 20. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986;7:177-88.
 21. Parsa A, Peden E, Lum RF, et al. Association of angiotensin-converting enzyme polymorphisms with systemic lupus erythematosus and nephritis: analysis of 644 SLE families. *Genes Immun* 2002;3:S42-6.
 22. Sato H, Akai Y, Iwano M, et al. Association of an insertion polymorphism of angiotensin-converting enzyme gene with the activity of systemic lupus erythematosus. *Lupus* 1998;7:530-4.
 23. Elkins JS, Douglas VC, Johnston SC. Alzheimer disease risk and genetic variation in ACE: a meta-analysis. *Neurology* 2004;62:363-8.
 24. Qu H, Lu Y, Lin S. Meta-analysis on the association of ACE/ID polymorphism and essential hypertension in Chinese population. *Zhonghua Yu Fang Yi Xue Za Zhi* 2001;35:408-11.
 25. Fujisawa T, Ikegami H, Kawaguchi Y, et al. Meta-analysis of association of insertion/deletion polymorphism of angiotensin I-converting enzyme gene with diabetic nephropathy and retinopathy. *Diabetologia* 1998;41:47-53.
 26. Sharma P. Meta-analysis of the ACE gene in ischaemic stroke. *J Neurol Neurosurg Psychiatry* 1998;64:227-30.
 27. Samani NJ, Thompson JR, O'Toole L, Channer K, Woods KL. A meta-analysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction. *Circulation* 1996;94:708-12.