Angiotensin-Converting Enzyme Gene Insertion/Deletion, not Bradykinin B2 Receptor -58T/C Gene Polymorphism, Associated With Angiotensin-Converting Enzyme Inhibitor-Related Cough in Chinese Female Patients With Non–Insulin-Dependent Diabetes Mellitus

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To investigate the genetic susceptibility associated with cough related to angiotensin-converting enzyme inhibitor (ACEI) therapy in patients with type 2 diabetes, 189 non–insulin-dependent diabetes mellitus (NIDDM) patients with proteinuria or hypertension treated with perindopril were studied. Cough was considered to be present if the patients had been bothered by a cough during treatment and if they had had related symptoms for at least 2 weeks without an identifiable cause. Polymerase chain reaction (PCR) coupled with single-strand conformation polymorphism (SSCP) was used to detect polymorphisms of ACE and bradykinin B2-receptor genes. After 8 weeks of treatment, 49.2% (93 of 189) of our NIDDM patients were found to be suffering from ACEI-related cough. ACEI-related cough was mainly associated with female patients, with 71.7% (76 of 106) of female and only 20.5% (17 of 83) of male patients experiencing cough after ACEI treatment. There was a significant association of ACE II genotype with ACEI-related cough. The genotype frequencies were 58.2% for II, 47.8% for ID, and 16.7% for DD in patients with ACEI-associated cough and 41.8% for II, 52.2% for ID, and 83.3% for DD in subjects without ACEI-associated cough ($\chi^2 = 10.268$; $df = 2$, $P = .006$). As female patients made up the majority of the subjects suffering from ACEI-related cough, we further analyzed the association of ACE I/D genotype with ACEI-related cough separately by sex. Male patients with ACEI-related cough were not associated with ACE I/D genotype distribution, while female patients were strongly associated with ACE I/D genotype polymorphism ($\chi^2 = 16.12$; $df = 2$; $P < .001$). There was no association between the bradykinin B2 receptor gene –58T/C polymorphism with ACEI-related cough. In conclusion, our results indicate that Chinese diabetic female subjects are susceptible to ACEI-related cough, and this susceptibility may be genetically predetermined.

PERHAPS THE MOST promising group of drugs used in the treatment of hypertension in patients with diabetic nephropathy are angiotensin-converting enzyme inhibitors (ACEI).[1] These agents do not alter lipid profile and thus do not adversely affect other cardiovascular risk factors as other agents do.[2] Furthermore, they appear to increase glucose disposal rate, enhance insulin sensitivity, and may improve glycemic control.[3] Also, these drugs have been reported to reduce protein excretion in azotemic patients and decrease the rate of decline of renal function in patients with diabetic nephropathy.[4,5] However, the coughing that results from ACEI therapy is a well-recognized complication[6,7] with the literature reporting the frequency of cough varying from 0.7% to as high as 53%.[8-11] A high incidence of cough has been found in Chinese populations.[10,11] Varying in severity, the dry cough is usually preceded by a tickling sensation in the back of the throat and appears to be more common in nonsmokers, women, and patients with renal failure.[12,13] The cause of the cough is reported to be intrinsic to the mechanism of action of ACEI, and so change to another ACEI is not recommended because of apparent cross-reactivity.[14] The accumulation of kinins has been suggested to play a major role in ACEI treatment-related cough. This accumulation probably results from the inhibition of the degradation of kinins, particularly bradykinin, in the airway, but the precise mechanism is still unknown. Furuya et al[15] have stated that a genetic predisposition has been found to be associated ACEI-related cough. Other studies have also implied a genetic predetermination of ACEI-related cough caused by specifically implicated variants of the genes that encode ACE, chymase, and bradykinin B2 receptors.[16-18]

Recently, we reported a high incidence of ACEI-related cough in postmenopausal diabetic Chinese women with hypertension or nephropathy, some patients experiencing intolerable cough-related stress incontinence.[19] To date, there has been no investigation of a possible genetic mechanism of the high incidence of ACEI-related cough in the Chinese population. To further clarify the possible genetic factors associated with this phenomenon, we designed a case-association study to investigate the relationship of the bradykinin B2 receptor and ACE insertion/deletion (I/D) gene polymorphism to ACEI-induced cough in Chinese subjects with non–insulin-dependent diabetes mellitus (NIDDM).

MATERIALS AND METHODS

Patients with NIDDM and hypertension or nephropathy who consecutively attended the diabetic clinic of Ping-Tung Christian Hospital were studied. The control subjects were apparently unrelated subjects who entered the health examination program of our hospital for a health evaluation. They received detailed interviews regarding personal disease history and smoking history. All study subjects were of Han Chinese origin, with no known ancestors of other ethnic origin, and living in the same region at the time of study. The diagnosis of type 2 diabetes was based on the World Health Organization (WHO) criteria.[20] All patients underwent complete physical examinations and routine biochemical analyses of blood and urine, as well as assessment of the presence and extent of macrovascular or microvascular diabetic

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complications. Hypertension was defined when patients had an office diastolic blood pressure (BP) of greater than 90 mm Hg on a mercury sphygmomanometer. Most of the hypertensive patients were untreated, and those who were receiving antihypertensive agents were asked to discontinue treatment for 2 weeks prior to the study. Patients who had had a diagnosis of urinary tract infection, urolithiasis, liver cirrhosis, bronchitis, emphysema, congestive heart failure, or chronic lung disease were excluded. Cough was considered to be present if they had been bothered by a cough during the treatment and if they had symptoms for at least 2 weeks without an identifiable cause, such as acute respiratory infection. Although a history of smoking was obtained for each patient, smokers were not excluded in this study. Seated BP, plasma biochemical parameters, and urinary microalbumin were measured after an overnight fast. Plasma triglycerides, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, uric acid, creatinine, and glucose were determined by standard commercial methods on a parallel-multichannel analyzer (Hitachi 7170A, Tokyo, Japan). The urinary albumin concentration was measured by immunoturbidmetry (Beckman Instruments, Galway, Ireland). The detection limit was 2 mg/L, and the interassay and intraassay coefficient of variance was less than 8%.

Patient visits were scheduled every 2 weeks during the study period. This study was approved by the human research review committee of our hospital, and informed consent was obtained from each patient. Patients received a single daily dose of perindopril 4 mg (Acertil; Les Laboratoires Servier Industrie, Gidy, France). The dosage of the patients' usual antihypertensive therapy could be increased or other non-ACEI antihypertensive therapy could be added if needed to achieve a target BP. The severity and frequency of cough were assessed by a visual analogue scale (VAS) at 2-week intervals.

Bradykinin B2 Receptor Gene –58Thymidine/Cytosine (T/C) Polymorphism

Genomic DNA was prepared from peripheral blood using standard techniques. The primers for the polymerase chain reaction (PCR) amplification were 5′-GCAGAGCTCAGCTGGAGGAG-3′, located in the promoter, and 5′-CCTCCTCGGAGCCCAGAAG-3′, located in the promoter/exon 1. Primers were designed from the bradykinin B2 receptor gene reported by Kammerer et al.[21] PCR was performed at a final concentration of 1 × PCR buffer (10 mmol/L Tris-HCl pH 8.3; 50 mmol/L KCl; 1.5 mmol/L $MgCl_2$), 0.2 mmol/L deoxynucleoside triphosphate (dNTP), 0.4 μmol/L sense and antisense oligos, and 2.0 U of Tag DNA polymerase (Takara Tag; Takara Shuzo, Otsu Shiga, Japan) in a total volume of 50 μL. After an initial denaturation step at 90°C for 3 minutes, the amplifications were performed for 30 cycles of 45 seconds at 94°C, 45 seconds at 60°C, and 90 seconds at 72°C, and a final extension time of 5 minutes at 72°C in a thermocycler (Gene Amp PCR System 9700, Perkin-Elmer, Foster City, CA). PCR products were subjected to a single-strand conformation polymorphism (SSCP) electrophoresis in a 20% polyacrylamide (2X Tris Hcl boric acid EDTA buffer [TBE]) gel. Electrophoresis was performed at 24°C at 180 V for 20 hours, and the gel was then silver-stained. Four representative samples of TT and TC genotype detected by SSCP were sequenced to confirm the thymidine or cytosine at nucleotide position –58 upstream from the putative transcription start site.

ACE I/D Polymorphism

For the ACE I/D polymorphism, the primer pairs used and the annealing temperature were: forward, 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and reverse, 5′-GATGTGGCCATCACATTCGTCAGAT-3′, amplifying the intron 16 region where the I/D fragment is located. PCR amplification products were obtained using 25-μL reactions (0.5 pg genomic DNA, 500 pmol of primers, 0.5 mmol/L each of deoxyadenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), thymidine triphosphate (TTP), and 1.5 mmol/L $MgCl_2$; 0.5 U Tag DNA polymerase (Takara); 50 mmol/L KCl; 0.001% gelatin; 10 mmol/L Tria-HcL pH 8.3) with 4-minute denaturation at 94°C, followed by 35 cycles of 15 seconds at 94°C, 5 seconds at 67°C, and 30 seconds at 74°C in a thermal cycler. Reaction was terminated at 72°C at 2 minutes. To avoid ID/DD mistyping of heterozygotes as DD-homozygotes, all of the DD-genotype samples were confirmed using a pair of primers that produce an amplified product only in the presence of the insertion and were used to verify the polymorphism, forward, 5′-TGGGACCACAGCGCCCGCCACTAC-3′, and reverse, 5′-TCGCCAGCCCTCCCATGCCCATAA-3′. The PCR reaction conditions were similar to the procedures used for I/D detection except that the annealing temperature was changed to 62°C. All PCR products were visualized after electrophoresis on a 2% agarose gel and ethidium bromide staining. Genotyping was performed in a blinded fashion.

Statistical Analysis

The data are shown as the mean ± SD. All statistical analyses were performed using the Statistical Package for Social Science (SPSS for Windows, version 7.5.1, 1996, SPSS, Chicago, IL) program. The χ^2 test was used to study the categorical variables. Other variables were compared using unpaired *t* test. Differences were considered statistically significant if there was a *P* value of $< .05$.

RESULTS

A total of 189 patients with hypertension or nephropathy (microalbuminuria or more advanced) receiving perindopril treatment completed the study. After 8 weeks of therapy, 49.2% (93 of 189) of our NIDDM patients were found to have ACEI-related cough. One noteworthy finding was that most subjects with ACEI-related cough were female patients. In this study, 71.7% (76 of 106) female patients and 20.5% (17 of 83) male patients were found to be experiencing cough after ACEI treatment (Table 1). Twenty female patients reported intolerable cough and cough-related urinary stress incontinence. Table 2 shows the demographic characteristics of patients with or without severe cough related to ACEI. Patients with ACEI-related cough were also found to be older than those patients without cough.

The ACE I/D genotype distributions in subjects with or without ACEI-associated cough are shown in Table 1 and Fig 1. The genotype frequencies were 58.2% for II, 47.8% for ID, 16.7% for DD in patients with ACEI-associated cough and 41.8% for II, 52.2% for ID, and 83.3% for DD in subjects without ACEI-associated cough, the II genotype being significantly associated with ACEI-related cough ($\chi^2 = 10.268$; $df = 2$; $P = .006$). As ACEI-related cough sufferers were mostly female, we further analyzed the association of ACE I/D genotype separately by sex. We found that male patients with ACEI-related cough were not associated with ACE I/D genotype distribution, while female patients were, and strongly so (Table 1, $\chi^2 = 16.12$; $df = 2$; $P < .001$).

Genotype distributions of the bradykinin B2 receptor gene -58T/C polymorphism in type 2 diabetic patients were 30.1% for CC, 42.2% for TC, and 27.7% for TT, respectively. There was no statistically significant difference found between the bradykinin b2 receptor gene polymorphism and ACEI-associated cough (58.3% for CC, 46.6% for TC, and 44.2% for TT, respectively, $\chi^2 = 2.317$; $df = 2$; $P = .314$).

The VAS was used to quantify the patients' perception of

Table 1. Distribution of ACE I/D Genotype in Control Subjects and Diabetic Patients With or Without ACEI-Related Cough

	Genotype				
	II n (%)	ID n (%)	DD n (%)	Total No.	*P* Value
Control subjects	100 (50.3)	81 (40.7)	18 (9.0)	199	
Male	50 (48.1)	45 (43.2)	9 (8.7)	104	
Female	50 (52.6)	36 (37.9)	9 (9.5)	95	
NIDDM	79 (41.8)	92 (48.7)	18 (9.5)	189	NS*
Male	32 (38.6)	43 (51.8)	8 (9.6)	83	
Female	47 (44.3)	49 (46.2)	10 (9.4)	106	
NIDDM with ACEI-related cough	46	44	3	93	
Male	7	9	1	17	
Female	39	35	2	76	
NIDDM without ACEI-related cough	33	48	15	96	.006
Male	25	34	7	66	.837
Female	8	14	8	30	.001

NOTE. Comparisons are performed by χ^2 test.

Abbreviation: NS, not significant.

*When compared with the control subjects.

frequency of cough. A very low VAS score (0.2 cm) was found at the beginning of the study with changes in VAS score being significantly higher in the patients with cough (3.3 ± 2.5 cm, $P < .01$) at the end of study (Table 2). The time of coughing associated with ACEI varied among patients, ranging from 2 days to 1 month (mean, 7.5 ± 5.5 days).

DISCUSSION

The current study shows that Chinese subjects with NIDDM receiving perindopril have a high prevalence (49.2%) of cough.

Table 2. Clinical Characteristics of Patients Studied

	NIDDM and ACEI-Related Cough	
Demography	With	Without
No. (%)	93 (49.2)	96 (50.8)
Age (yr)	63.3 ± 8.3	58.6 ± 11.3†
M/F	17/76	66/30†
BMI	26.1 ± 3.6	25.7 ± 3.5
WHR	0.95 ± 0.06	0.95 ± 0.06
Known diabetes duration (yr)	8.9 ± 6.6	8.3 ± 6.4
Baseline BP		
SSBP (mm Hg)	160.4 ± 17.3	165.7 ± 17.9
SDBP (mm Hg)	94.0 ± 9.3	93.8 ± 11.4
Endpoint BP		
SSBP (mm Hg)	151.2 ± 17.2*	151.2 ± 17.2*
SDBP (mm Hg)	89.5 ± 12.3*	92.8 ± 12.0*
Cholesterol (mg/dL)	210.6 ± 45.6	204.5 ± 58.5
Triglyceride (mg/dL)	204.8 ± 140.1	180.6 ± 135.1
Uric acid (mg/dL)	6.5 ± 1.87	6.9 ± 2.0
Creatinine (md/dL)	1.3 ± 1.0	1.3 ± 0.7
HbA_1C (%)	8.5 ± 1.9	8.3 ± 2.0
VAS		
Baseline	0.23 ± 0.19	0.24 ± 0.13
Endpoint	1.29 ± 1.26*	3.29 ± 2.53*†

NOTE. Data are mean ± SD.

Abbreviations: BMI, body mass index; WHR, waist to hip ratio; VAS, visual analogue scale.

*$P < .01$ when compared with the baseline data.

†$P < .01$ when compared between patient groups.

Prevalence estimates of ACEI cough vary widely among previous studies. Early reviews noted a frequency rate as low as 1% to 2%, whereas recent literature has disclosed it to be as high as 15% to 53%.[7-12] A high incidence of cough associated with ACEI has been found among African-Americans and Chinese,[11,22] and it appears to be more common in nonsmokers, women, and patients with renal failure.[12,13] In 1 case-control study in a Chinese population, the incidence of persistent cough in patients taking ACEIs was 44% compared with 11.1% in controls not taking ACEIs.[11] In a study comparing ACEI-associated cough in Hong-Kong Chinese and Auckland Caucasians, the incidence of cough in ACEI-users versus non-ACEI-users was 53% and 10% for Chinese and 18% and 5%

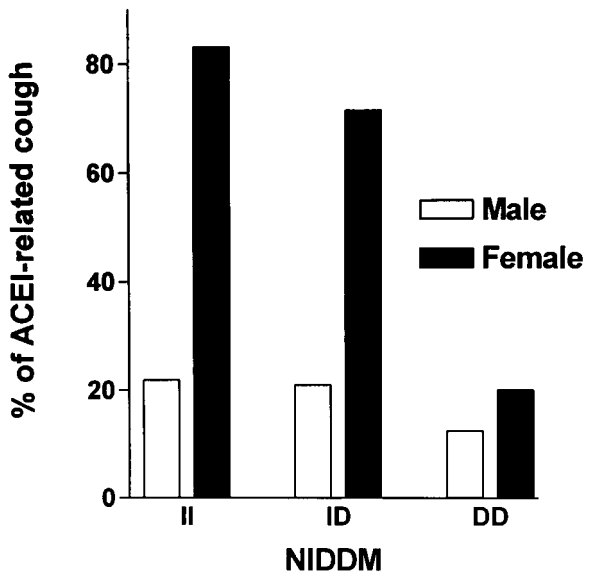

Fig 1. Prevalence of investigator-assessed definite cough during an entire 8-week treatment period. Diabetic female patients with II genotype receiving perindopril had a significantly higher incidence of cough when compared with the other groups, $\chi^2 = 16.12$; $df = 2$; $P < .001$.

for Caucasians.[11] Our study also showed that the ACEI-related cough mainly appeared in female patients. The reasons for this female predominance cannot be well explained, although Isarili and Hall[9] have postulated that women have a lower cough threshold and, therefore, may report this adverse effect more often.

ACEI is the drug of choice in patients with nephropathy.[1] It can reduce intraglomerular pressure, inhibit mesangial cell proliferation and matrix production, and lower BP, contributing to the protective mechanism of progression of diabetic nephropathy. However, the high incidence of ACEI-related cough in the Chinese population limits the use of ACEI in this population. Such a troublesome side effect often causes these patients intolerable distress and sleep disturbance. To date, discontinuation of the medication has been the only way to resolve this problem. Being able to identify subjects vulnerable to this side effect prior to the treatment would allow us to avoid this unpleasant effect and increase patient compliance with their medical therapy.

It has been found that an insertion/deletion polymorphism of ACE gene affects the serum ACE level,[23] and ACE gene polymorphism has been known to contribute to the ethnic differences in response to ACEI treatment.[24] In Caucasians, 44% of the variance in circulating ACE activity is accounted for by genetic polymorphism,[25] and plasma ACE activity has also been found to be genetically determined in the Chinese population.[26] ACE inactivates bradykinin, substance P, and neurokinin A, all potent bronchoconstrictors and inflammatory mediators. Substance P and neurokinin are potent inducers of airway smooth muscle constriction, bronchial edema, extravasation of plasma, and mucus hypersecretion, acting as important mediators of neurogenic inflammation.[27] Using aerosols of distilled water for testing, Morice et al[16] found the ACE genotype to be a link between ACE activity and the cough reflex. Patients with ACE II genotype may have lower serum or tissue levels of ACE than other genotype patients. The decreased levels of ACE might result in less inactivation of bradykinin, substance P, and neurokinin, thereby accounting for the increased bronchial responsiveness and susceptibility to cough.

In a meta-analysis of studies composed mostly of Caucasians, the distribution of the I/D polymorphism was found to be 22.7% II, 49% ID, and 28.3% DD, with the ACE levels of DDs about twice that of IIs.[28,29] The prevalence of I allele is much higher in Chinese than in Caucasians, and fewer Chinese have the DD genotype (0.70 among Chinese compared with 0.43 in the Caucasian population).[25,30] This fact may explain the high prevalence of cough associated with ACEI treatment in the Chinese population.

Recently, Mukae et al[18] reported that bradykinin B2 receptor gene –58T/C polymorphism is associated with ACEI-related cough, especially in female patients. Using promoter assay studies of genetic variants of the bradykinin receptor, -58T was found to have a higher transcriptional rate than that of –58C,[21,31] and it has been suggested that the transcriptional activity of the promoter might be involved in the appearance of ACEI-related cough.[18] The current study reveals no association between the bradykinin B2 receptor –58T/C polymorphism and ACEI-related cough in Chinese NIDDM patients and may suggest that ACE activity, rather than the bradykinin B2 receptor transcriptional rate, be the main determinant of ACEI-related cough.

In conclusion, our results indicate that Chinese diabetic female subjects are susceptible to ACEI-related cough, and this susceptibility may be genetically predetermined.

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REFERENCES

1. Guidelines Subcommittee: 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. J Hypertens 17:151-183, 1999

2. Keilani T, Schlueter W, Batlle D: Selected aspects of ACE inhibitor therapy for patients with renal disease: Impact on proteinuria, lipids and potassium. J Clin Pharmacol 35:87-97, 1995

3. Pollare T, Lithell H, Berne C: A comparison of the effects of hydrochlorothiazide and captopril on glucose and lipid metabolism in patients with hypertension. N Engl J Med 321:868-873, 1989

4. Hollenberg NK, Raij L: Angiotensin-converting enzyme inhibition and renal protection. An assessment of implications for therapy. Arch Intern Med 153:2426-2435, 1993

5. Nielsen FS, Rossing P, Gall MA, et al: Impact of lisinopril and atenolol on kidney function in hypertensive NIDDM subjects with diabetic nephropathy. Diabetes 43:1108-1113, 1994

6. Coulter DM, Edwards IR: Cough associated with captopril and enalapril. BMJ 294:1521-1523, 1987

7. Goldszer RC, Lilly LS, Solomon HS: Prevalence of cough during angiotensin-converting enzyme inhibitor therapy. Am J Med 85:887, 1988

8. Yeo WW, Ramsay LE: Persistent dry cough with enalapril: Incidence depends on method used. J Hum Hypertens 4:517-520, 1990

9. Israili ZH, Hall WD: Cough and angioneurotic edema associated with angiotensin-converting enzyme inhibitor therapy. A review of the literature and pathophysiology. Ann Intern Med 117:234-242, 1992

10. Woo J, Chan TY: A high incidence of cough associated with combination therapy of hypertension with isradipine and lisinopril in Chinese subjects. Br J Clin Pract 45:178-180, 1991

11. Woo KS, Nicholls MG: High prevalence of persistent cough with angiotensin converting enzyme inhibitors in Chinese. Br J Clin Pharmacol 40:141-144, 1995

12. Punzi HA: Safety update: Focus on cough. Am J Cardiol 72: 45H-48H, 1993

13. Os I, Bratland B, Dahlof B, et al: Female sex as an important determinant of lisinopril induced cough. (letter) Lancet 339:372, 1992

14. Bucknall CE, Neilly JB, Carter R, et al: Bronchial hyperreactivity in patients who cough after receiving angiotensin converting enzyme inhibitors. BMJ 296:86-88, 1988

15. Furuya K, Yamaguchi E, Hirabayashi T, et al: Angiotensin-I-converting enzyme polymorphism and susceptibility to cough. Lancet 343:354, 1994

16. Morice AH, Turley AJ, Linton TK: Human ACE gene polymorphism and distilled water induced cough. Thorax 52:111-113, 1997

17. Zee RY, Rao VS, Paster RZ, et al: Three candidate genes and angiotensin-converting enzyme inhibitor-related cough. A pharmacogenetic analysis. Hypertension 31:925-928, 1998

18. Mukae S, Aoki S, Itoh S, et al: Bradykinin B2 receptor gene polymorphism is associated with angiotensin-converting enzyme inhibitor-related cough. Hypertension 36:127-131, 2000

19. Lee YJ, Chiang YF, Tsai CR: Severe nonproductive cough and cough-induced stress urinary incontinence in diabetic postmenopausal women treated with ACE inhibitor. Diabetes Care 23:427-428, 2000

20. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus, provisional report of a WHO consultation. Diabet Med 15:539-553, 1998

21. Kammerer S, Braun A, Arnold N, et al: The human bradykinin $\beta 2$ receptor gene: Full length cDNA, genomic organization and identification of the regulatory region. Biochem Biophys Res Commun 211:226-233, 1995

22. Elliott WJ: Higher incidence of discontinuation of angiotensin converting enzyme inhibitors due to cough in black subjects. Clin Pharmacol Ther 60:582-588, 1996

23. Rigat B, Hubert C, Alhenc-Gelas F, et al: An insertion/deletion polymorphism in the angiotensin-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 86:1343-1346, 1990

24. Ding PYA, Hu OYP, Pool PE, et al: Does Chinese ethnicity affect the pharmacokinetics and pharmacodynamics of angiotensin-converting enzyme inhibitors? J Hum Hypertens 14:163-170, 2000

25. Tiret L, Rigat B, Visvikis S, et al: Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. Am J Hum Genet 51:197-205, 1992

26. Chiang FT, Lai ZP, Chern TH, et al: Lack of association of the angiotensin converting enzyme polymorphism with essential hypertension in a Chinese population. Am J Hypertens 10:197-201, 1997

27. Nadel JA: Neurogenic inflammation in airways and its modulation by peptidase. Ann N Y Acad Sci 664:408-414, 1992

28. Samani NJ, Thompson JR, O'Toole L, et al: A meta-analysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction. Circulation 94:708-712, 1996

29. Singer DR, Missouris CG, Jeffery S: Angiotensin-converting enzyme gene polymorphism. What to do about all the confusion? Circulation 94:236-239, 1996

30. Lee EJ: Population genetics of the angiotensin-converting enzyme in Chinese. Br J Clin Pharmacol 37:212-214, 1994

31. Braun A, Kammerer S, Maier E, et al: Polymorphism in the gene for the human $\beta 2$-bradykinin receptor: New tools assessing a genetic risk for bradykinin-associated diseases. Immunopharmacology 33:32-35, 1996