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Possible Association of ACE Gene I/D Polymorphism With Blood Pressure——Lowering Response to Hydrochlorothiazide

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Objective To explore the association between polymorphism in the ACE I/D gene and blood pressure-lowering response to hydrochlorothiazide (HCTZ) in 829 patients. **Methods** HCTZ 12.5 mg was taken once a day for six weeks. The blood pressure reduction and ratio reaching target blood pressure were compared in different ACE genotype groups. **Results** The reduction in SBP of patients carrying DD was greater than that in other groups carrying II or ID (12.2 mmHg versus 5.4 mmHg, 12.2 mmHg versus 4.4 mmHg, respectively, $P<0.05$). The reduction in MAP of patients carrying DD was also greater than that in other groups carrying II or ID (6.9 mmHg versus 3.9 mmHg, 6.9 mmHg versus 3.6 mmHg, respectively, $P<0.05$). The ratio reaching target blood pressure in DD groups was significantly higher than that in II or ID groups ($P<0.05$). The pre-treatment SBP, DD genotype, aldosterone levels entered the multi-linear regression model significantly and might affect the reduction of SBP. The pre-treatment DBP, aldosterone levels, DD genotype entered the multi-linear regression model significantly and might affect the reduction of DBP. The pre-treatment MAP, DD genotype, aldosterone levels entered the multi-linear regression model significantly and might affect the reduction of MAP. **Conclusion** ACE genotyping is associated with blood pressure-lowering response to HCTZ. Specific genotypes might be associated with the response to specific antihypertensive treatment.

Key words: Hypertension; Peptidyl-dipeptidase A; Genotype; Treatment

INTRODUCTION

Essential hypertension is a multi-factorial disease, associated with the interaction of genetic and environmental factors. Recently, a number of large –scale randomized clinical trials have demonstrated that hydrochlorothiazide (HCTZ) could decrease not only blood pressure but also the morbidity and mortality of stroke and coronary heart disease. However, inter-individual variation in response to HCTZ can be found in different hypertensive patients. It has long been suspected that inter-individual variation in drug responses may be influenced by genetic factors[1]. Recently, many clinical trials have found that patients carrying three different genotypes of ACE have different responses to antihypertensive drug therapy. Therefore, we hypothesized that ACE gene I/D polymorphism might be associated with the antihypertensive efficacy of HCTZ. Knowledge of polymorphous variation in ACE gene helps to predict blood pressure responses to antihypertensive drug therapy in individual patients and may also provide new insights into molecular mechanisms responsible for elevation of blood pressure.

MATERIALS AND METHODS

Study Design

The present study was conducted in Tangshan City, Hebei Province of China. Seven hospitals, including Kailuan Hospital affiliated to North China Coal Mining Medical College, took part in the single blinded clinical trials. The patients were screened in mining districts of Kailuan Coal Mining Industry Group for four weeks and the eligible patients were enrolled into the study after signing their informed consent. The eligible criteria were as following: ① male or female patients at the age above eighteen; ② According to the guidelines of 1999 WHO/ISH, mild or moderate hypertensive patients (office SBP

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between 140-179 mmHg, and/or DBP between 90-109 mmHg in at least three consecutive measures at weekly intervals). Patients with secondary hypertension, severe hepatic disorders or nephropathy, severe cardiovascular disease, and patients allergic to sulfa were excluded by medical history, physical examination, laboratory tests and other tests if indicated. Potentially eligible individuals entered a 2-week run-in period, during which they received open-labelled placebo. Participants who adhered to the run-in therapy with placebo were ordered to continuously take HCTZ (12.5 mg, once a day). The patients were also advised to take medicine at the same time everyday, and stop taking it before the follow-up day. Finally, a total of 829 eligible patients were registered in this study. The study was approved by the Kailuan Hospital Ethnic Committee.

Data-collection and Follow-up

After a 2-week run-in period, information about medical history, smoking habits, alcohol intake and current medication was collected. Venous blood was sampled after an overnight fast in sitting position, for the measurement of plasma rennin activity and total cholesterol and genotyping. All biochemical measurements were performed in the Laboratory Center of Kailuan Hospital (Tangshan, China), which fulfilled the quality control criteria of the regulatory authority of Hebei Province. Blood urinary nitrogen, creatinine, total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, very low-density lipoprotein, urine acid, aldosterone levels, Ang Ⅱ and plasma rennin activity were measured before and after the study. Based on the information obtained, the case report form was filled up.

Blood pressure was measured at least three times at an interval of five minutes in accordance with the procedures recommended by 1999 WHO/ISH guidelines. Then three measurements were accomplished on average. The BP measurements were always performed by the same doctor between 8:00 and 10:00 a. m., about 24 hours after the last HCTZ dosage.

DNA Analysis

Venous blood samples were collected in EDTA for extraction of DNA from buffy coats using a hydroxybenzene-chloroform procedure. ACE I/D polymorphism was detected as previously described[2]. All samples genotyped as DD underwent a repeated polymerase chain reaction (PCR) using insertion-specific primers. PCR conditions were as before except for an annealing temperature of 67℃. The sense primer was 5'-TGGGACCACAGCGCCCGCCACTAC-3', and the antisense primer was 5'-TCGCCAGCCCTCCCATGCCCATAA-3', respectively. In the present study, the ratio of mistyping DD genotype reached about 7%, similar to the results from other studies based on the Chinese population[3].

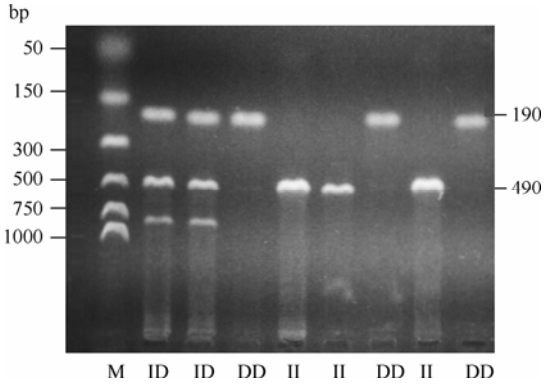

FIG.1. Determination of ID, II, and DD genotypes by PCR.

Statistical Analysis

Figures were made with Excel-2000 software and analyzed with SPSS version 11.0. Baseline characteristics were compared between 3 genotypes by χ^2 test (for categorical variables) or ANOVA (for continuous variables). Hardy-Weinberg equilibrium was tested by χ^2 test. Differences between 3 genotypes in reduction of blood pressure were investigated by ANOVA. The multi-linear stepwise regression was made to assess the factors responsible for the reduction of blood pressure. $P<0.05$ was considered statistically significant.

RESULTS

Study Population and Baseline Characteristics

Baseline demographic characteristics are presented in Tables 1-3. A total of 829 eligible patients were enrolled in this study. Among them, 20 were dropped from the study because of loss of follow-up, and genotype was not detected in 4. Finally, data obtained from 805 patients including 456 males (56.7%) and 349 females (43.3%) with full records were analyzed. The mean age was 56 (56.8±9.4) years. The baseline SBP and DBP were (150.9±14.8) mmHg and (93.2±8.8) mm Hg, respectively. II, ID, and DD genotypes were found in 326 (40.5%), 320 (39.7%), and 159 (19.8%) patients, respectively. There was no significant difference in the distribution by gender, age, and life style between the three genotypes ($P>0.05$) (Tables 1 and 2). The ACE and aldosterone levels of DD genotype were the

highest in the three groups (P<0.05) (Table 3). No significant difference in other clinical biochemical measurements between the three groups was found (P>0.05).

TABLE 1

Distribution of ACE Genotype by Gender and Age

ACE	Male				Total	Female				Total
	<50	50-59	60-69	⩾70		<50	50-59	60-69	⩾70	
II	34	78	49	33	194	26	69	32	5	132
ID	49	66	43	17	175	22	68	49	6	145
DD	18	26	28	15	87	14	31	23	4	72
Total	101	170	120	65	456	62	168	104	15	349

Note. χ^2=1.829, P=0.401 by gender; χ^2=9.451, P=0.145 by age.

TABLE 2

Comparison of Drug History and Life Behavior Among ACE Genotypes

Item	ACE Genotypes			Total	χ^2 Value	P Value
	II	ID	DD			
Salt Preference						
Mild	100	102	49	251		
Moderate	154	148	67	369	2.135	0.711
Heavy	72	70	43	185		
Physical Activity						
None	121	99	50	270		
Seldom	116	140	72	328	6.451	0.168
Often	89	81	37	207		
Alcohol						
Yes	87	83	37	207	0.667	0.716
No	239	237	122	598		
Smoking						
Yes	74	70	27	171	2.216	0.330
No	252	250	132	634		
Drug Use History						
Yes	27	42	21	90	4.634	0.099
No	299	278	138	715		
Total	326	320	159	805		

TABLE 3

Comparison of Laboratory Examinations Among Different ACE Genotypes ($\bar{x} \pm s$)

Index	ACE Genotypes		
	II (n=326)	ID (n=320)	DD (n=159)
BUN (mmol/L)	5.78±1.95	5.74±1.86	6.10±1.94
Cr (umol/L)	1.54±16.82	88.59±17.20	90.18±18.58
GLU (mmol/L)	5.78±2.17	5.65±1.87	5.65±1.83
K^+ (mmol/L)	4.61±0.68	4.62±0.68	4.59±0.65
Na^+ (mmol/L)	143.17±6.13	142.43±5.62	143.20±5.23
UA (umol/L)	308.51±101.11	307.33±95.17	308.58±79.98
TG (mmol/L)	1.85±1.36	1.86±1.18	1.88±1.11
TC (mmol/L)	6.08±1.27	6.06±1.27	5.99±1.15
HDL-C (mmol/L)	1.50±0.49	1.54±0.42	1.45±0.41
LDL-C (mmol/L)	3.60±1.19	3.64±1.13	3.64±1.13
VLDL-C (mmol/L)	0.24±0.53	0.21±0.41	0.22±0.41
PRA (ng·mL·h)	1.87±1.49	1.87±1.54	1.68±1.46
ACE (U/L)	31.94±15.29	41.48±16.68[a]	47.94±19.77[b]
AT Ⅱ (pg/mL)	65.95±32.09	65.17±29.91	63.54±20.04
ALD (ng/L)	150.56±52.75	152.57±49.68	168.91±42.80[b]

Note. [a]P<0.05 *vs* II group; [b]P<0.05 groups with II, ID.

Different Reduction of Blood Pressure in Three Groups

As shown in Table 4, the blood pressure decreased significantly after six weeks of treatment (P<0.05). The decrease in SBP of the patients carrying DD was greater than that in other groups carrying II or ID (12.2 mmHg versus 5.4 mmHg, 12.2 mmHg versus 4.4 mmHg, respectively, P<0.05). The decrease in MAP of patients carrying DD was greater than that in other groups carrying II or ID (6.9 mmHg versus 3.9 mmHg, 6.9 mmHg versus 3.6 mmHg, respectively, P<0.05). No significant difference in the decrease of DBP was found in three groups.

Ratio Reaching Target Blood Pressure in Different Genotypes Groups

The ratio of target blood pressure in groups with different genotypes is shown in Table 5. The total proportion of patients whose blood pressures reached the target in three genotypes was 23.1%, and the ratio of target blood pressure in DD groups was higher than that in II and ID groups (P<0.05).

Factors Responsible for the Reduction of Blood Pressure

As shown in Table 6, the pre-treatment SBP, DD genotype, aldosterone levels entered the multi-linear regression model significantly and might affect the decrease of SBP. As shown in Table 7, the pre-treatment DBP, aldosterone levels, DD genotype entered the multi-linear regression model significantly and might affect the decrease of DBP. As shown in Table 8, the pre-treatment MAP, DD genotype, aldosterone levels entered the multi-linear regression model.

TABLE 4

Comparison of Reduction of BP Among Different ACE Genotypes ($\bar{x} \pm s$)

BP (mmHg)	ACE Genotypes			F	P
	II	ID	DD		
BSBP	149.1±14.4	151.1±14.0	154.2±15.2	6.04	0.003[a]
ASBP	143.6±14.8	146.7±14.0	141.9±15.8	0.45	0.639
△SBP	5.4±14.2	4.4±14.8	12.2±15.0	4.52	0.011[a]
BDBP	92.9±8.7	93.3±8.4	93.4±9.2	1.71	0.181
ADBP	89.7±8.3	90.1±8.8	89.0±8.3	0.15	0.860
△DBP	3.1±9.5	3.2±8.5	4.3±8.7	1.35	0.260
BMAP	111.6±8.7	112.6±9.8	113.6±8.9	3.63	0.027[a]
AMAP	107.7±9.3	109.0±9.9	107.2±9.4	0.33	0.721
△MAP	3.9±9.2	3.6±9.2	6.9±9.7	3.27	0.039[a]

Note. [a]P<0.05 *vs* groups with II and ID. BSBP represents baseline SBP, △SBP= ASBP-BSBP.

TABLE 5

Comparison of Rate of Target Blood Pressure in Different Groups

ACE Genotype	Target BP Group		Non-target BP Group		Total
	Number	%	Number	%	
II	68	20.9	258	79.1	326
ID	62	19.4	258	80.6	320
DD	56	35.2	103	64.8	159
Total	186	23.1	619	76.9	805

Note. χ^2=16.567, P<0.001.

TABLE 6

Variables and Parameters Affecting the Reduction of SBP

Variables	Partial Regression Coefficients	Standard Regression Coefficients	t Value	P Value
Constant	-59.421		-11.850	<0.001
SBP	0.458	0.454	14.777	<0.001
DD	5.776	0.154	4.994	<0.001
ALD	0.029	0.089	2.908	0.004

Note. F=91.688, P<0.001; adjusted R^2=0.253.

TABLE 7

Variables and Parameters Affecting the Reduction of DBP

Variables	Partial Regression Coefficients	Standard Regression Coefficients	*t* Value	*P* Value
Constant	-43.169		-13.717	<0.001
DBP	0.523	0.550	16.353	<0.001
ALD	0.015	0.088	2.588	0.010
DD	1.864	0.081	2.380	0.018

Note. F=94.179, P<0.001; adjusted R^2=0.316.

TABLE 8

Variables and Parameters Affecting the Reduction of MAP

Variables	Partial Regression Coefficients	Standard Regression Coefficients	*t* Value	*P* Value
Constant	-45.840		-11.744	<0.001
MAP	0.469	0.489	14.134	<0.001
DD	3.639	0.150	4.306	<0.001
ALD	0.020	0.108	3.118	0.002

Note. F=79.737, P<0.001; adjusted R^2=0.280.

DISCUSSION

Large-scale random clinical trials have demonstrated that HCTZ could decrease not only blood pressure but also the morbidity and mortality of stroke and coronary heart disease[4-5]. The important role of HCTZ in the antihypertensive therapy for hypertensive patients has been reiterated in the seventh report of the Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure[6]. However, an increasing number of studies have indicated that patients from different ethnic groups of population have different responses to HCTZ[7-8]. In the present study，the fact that only 23.1% of patients reached the target blood pressure also reiterated the discrepancy in antihypertensive efficacy of HCTZ.

Common variation in the ACE gene structure is defined by the insertion (*I*) or deletion (*D*) of 287 bp of nonsense DNA in intron 16, resulting in 3 genotypes (DD, ID, II). The ACE I/D polymorphism has been presumed to link disequilibrium with functional variants that determine enzyme activity. The DD genotype has a higher level of ACE activity than II and ID genotypes[9]. Starvroulaki[10] found that the decrease of systolic and diastolic blood pressure in patients carrying DD genotype was greater than that in those carrying II and ID after taking fosinopril for six weeks. A similar conclusion was drawn by a study based on the Chinese population[11]. These results suggest that the ACE genotypes might be associated with the antihypertensive efficacy of HCTZ. In our study, the decrease of SBP in patients carrying DD genotypes was significantly greater than that in those carrying II and ID genotypes. The patients carrying DD genotype had a higher blood pressure than those carrying the other two genotypes, indicating that the patients carrying DD genotype might have a better response to antihypertensive treatment with HCTZ. Multi-linear regression analysis showed that the DD genotype, baseline ALD and serum sodium levels might be one of the factors affecting the decrease of systolic blood pressure. However, a study on the Italian population has demonstrated that patients carrying II genotype have a better response to HCTZ than those carrying the other two genotypes. At present, the cause leading to this discrepancy in the results is still unknown. However, the comparable small sample of their study or different genetic backgrounds might be responsible, at least in part, for this conflict[12].

ACE, an important component of rennin angiotensin system, can catalyze AgⅠto produce AgⅡ. The latter can stimulate the synthesis of aldosterone, which can take part in regulation of blood pressure by influencing the metabolism of water and salt. The ACE activity in patients carrying DD genotype is higher than that in those carrying the other two genotypes. Therefore, their levels of AgⅡand aldosterone should be higher than those in patients carrying II and ID genotypes in theory. However, the present study only showed that patients carrying the DD genotype had a higher level of aldosterone than those carrying II and ID genotypes. No significant difference in the levels of AgⅡ was found in the three groups. The possibility that the concentration of AgⅡ in patients carrying DD genotype in local surroundings was higher than that in those carrying II and ID genotypes could not be ruled out. The hypothesis that the concentration of AgⅡ in patients carrying DD genotype in local surroundings is higher than that in those carrying II and ID genotypes may lead to the fact that the increasing level of aldosterone in patients carrying

DD genotype would result in excessive re-absorption of water and sodium. HCTZ can lower the blood pressure through two mechanisms. One pathway is involved in reduction of cardiac output by decreasing the volume of blood and extra-cellular fluids due the increased excretion of sodium. The other pathway is to lower the level of sodium in small arterial smooth muscle. Therefore, patients carrying DD genotype have a better response to HCTZ therapy than those carrying either of the other two genotypes.

Patients with lower rennin and salt sensitivity and higher blood pressure which are closely correlated with the vascular contraction induced by sodium ion and vascular volume, have a good response to diuretics. Although no significant difference in PRA between the three genotypes was found, patients carrying DD genotype with the lowest level of PRA in addition of the highest aldosterone level had a better response to HCTZ than those carrying II and ID genotypes, suggesting that DD genotype may be the biomarker of hypertensive patients with lower rennin level and salt sensitive hypertension, which was different from results of a study conducted in Japan[13].

In conclusion, the effect of different ACE genotypes on HCTZ antihypertensive efficacy may offer some clinical clues to the individual therapy for hypertension. Many other genetic factors such as α-aducin may be associated with the response to diuretics. Further studies should focus on the effect of genotypes responsible for the metabolisms of water and sodium on diuretics.

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