Journal of Genetics (2019) 98:1
https://doi.org/10.1007/s12041-018-1053-2

RESEARCH ARTICLE

Association of polymorphisms of *CYP11B2* gene –344C/T and *ACE* gene I/D with antihypertensive response to angiotensin receptor blockers in Chinese with hypertension

HONGTAO GONG*, LIYING MU, TONG ZHANG, XIUYING XU and FENGHE DU

Department of Cardiology, Beijing Tiantan Hospital, Capital University of Medical Science, Beijing, People's Republic of China
*For correspondence. E-mail: donnag@163.com.

Received 25 February 2018; accepted 27 September 2018; published online 30 January 2019

Abstract. The aim of this study was to determine whether the polymorphism of aldosterone synthase (*CYP11B2*) –344C/T and angiotensin-converting enzyme (*ACE*) insertion/deletion (I/D) were associated with the response of blood pressure (BP) to telmisartan treatment. After a two-week single-blind placebo run-in period, 148 patients with mild-to-moderate primary hypertension received monotherapy of telmisartan with 80 mg/day and then were followed up for eight weeks. Polymorphisms of *CYP11B2* –344C/T and *ACE* I/D gene were determined through polymerase chain reaction-restriction fragment polymorphism analysis. The relationship between these polymorphisms and changes in BP was monitored and evaluated after eight weeks of treatment. With respect to the polymorphism of *CYP11B2* –344C/T, the reduction in diastolic BP was significantly greater in patients carrying the C allele (CC+CT) compared with those carrying the TT genotype. There was no significant differences between *ACE* I/D polymorphism and BP reduction after treatment. We concluded that the aldosterone synthase –344C/T polymorphism was related to the antihypertensive treatment with telmisartan in hypertensive patients.

Keywords. essential hypertension; aldosterone synthase; angiotensin-converting enzyme; single-nucleotide polymorphism; angiotensin receptor blocker.

Introduction

Essential hypertension is a complex syndrome determined by both genetic and environmental factors. The response of patients to antihypertensive treatment is diverse (Materson 2004). Past effort to identify responders to therapy include phenotypic (age, race) (Preston *et al.* 1998) and biochemical (rennin profile, insulin sensitivity) (Laragh *et al.* 1988; Lind *et al.* 1995) methods, but none of these have found extensive clinical utility. The advent of the Human Genome Project (Venter *et al.* 2001) has generated rejuvenated interest in the pursuit of pharmacologic therapy targeted to individuals genetically identified as most likely to benefit from treatment. The rennin–angiotensin–aldosterone system (RAAS) plays a central role in the modulation of blood pressure (BP). Genetic variation in the genes encoding products of the RAAS has been studied extensively as candidate genes for essential hypertension (EH), such as *ACE* I/D gene (Patnaik *et al.* 2014), *ATIR* gene (Liu *et al.* 2015), *CYP11B2* gene (Alvarez-Madrazo *et al.* 2013), *AGT* gene (Kim *et al.* 2015) and so forth. It is reasonable to hypothesize that the RAAS gene polymorphism may be predictive of variation in BP response. Variant in genes of the RAAS has been investigated to influence the therapeutic responsiveness to antihypertensive drugs. However, the results of other studies are controversial (Taverne *et al.* 2010; Li *et al.* 2012; Do *et al.* 2014; Gupta *et al.* 2015).

Angiotensin receptor blockers (ARBs) are a kind of widely used antihypertensive drugs. ARBs act directly on the angiotensin II type 1 receptor to block the vasoconstrictive response to angiotensin II and inhibit angiotensin II-stimulated release of aldosterone. The aim of this study was to investigate the association between the *CYP11B2* –344C/T and *ACE* I/D polymorphism and the BP response to angiotensin II type 1 receptor blocker telmisartan in Chinese hypertensive patients.

Methods

Study subjects

In this study both male and female Chinese Han patients who met the following criteria were included: aged 18–80 years; history of essential hypertension; diastolic blood pressure (DBP) greater than or equal to 95 mmHg and lower than or equal to 109 mmHg; systolic blood pressure (SBP) lower than 180 mmHg. The exclusion criteria were as follows: secondary hypertension, congestive heart failure, cerebrovascular accident, myocardial infarction within the past three months; a documented history of unstable angina pectoris within the past three months; any clinically important abnormal laboratory findings, such as alanine aminotransferase (ALT) or creatinine level twice the upper limit of normal; pregnant or lactating in women. This study was approved by the appropriate Ethics Committees. All of the participating patients gave informed consent before any study procedures.

Study design

BP was measured by a well-trained doctor or nurse using a mercury sphygmomanometer after the patient had rested for at least 10 min in a seated position and was determined as the mean of three measurements taken 2 min apart. In this study, a total of 164 patients were recruited with essential hypertension. All antihypertensive agents were withdrawn before the start of a two-week placebo period. At the end of the placebo period, 11 patients were excluded and a total of 153 qualified patients were given telmisartan, 80 mg orally, once daily for eight weeks. Five patients were withdrawn from the study because of failing to follow up. Thus, 148 patients completed the eight-week trial, and their data were used for the present study. Blood was collected from all patients for genotype analysis.

Definition of study variables

Baseline information on the following variables was included in the analysis: gender, age, body mass index (BMI kg/m^2), BP, heart rate (HR), laboratory variables included the serum levels of ALT, creatinine, glucose, uric acid, total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL), sodium, potassium and angiotensin II.

Detection of CYP11B2 gene polymorphisms

Genomic DNA was isolated from peripheral leucocytes separated from the blood. The *CYP11B2* –344C/T polymorphism was determined through PCR/RFLP. PCR reaction volume of 20 μL contained 10 × PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.5 μM of each primer, 1 unit of Taq enzyme (Takara) and 100 ng of genomic DNA. The sequence of the sense oligonucleotide primer was 5-ATGTTGACCACCAGGAGGAGAC-3, and that of the antisense primer was 5-CCAGGGCTGAGAGGAGTAAAATG-3. The PCR cycling conditions consisted of initial denaturing step at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 62°C for 30 s and 72°C for 30 s, then a final elongation step at 72°C for 7 min. The PCR products were then digested overnight with 5 units of *Hae*III (New England Biochemicals), as described previously (Kupari *et al.* 1998), before separation on a 3% agarose gel and visualization with ethidium bromide. The amplification was cut into 273-bp as the –344 T allele and of 214 bp and 59 bp as the –344C allele after *Hae*III digestion.

Detection of ACE I/D gene polymorphisms

The genotype of the *ACE* gene was determined according to the method of Tiret *et al.* (1992). A 287-bp insertion or deletion polymorphism in intron 16 of the *ACE* gene was identified by the PCR method. The PCR reaction volume of 20 μL contains 10 × PCR buffer, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.5 μM of each primer, 1 unit of *Taq* enzyme (Takara) and 100 ng of genomic DNA. The sequence of the sense oligonucleotide primer was 5-CTGGAGACCACTCCATCCTTTCT-3 and that of the antisense primer was 5-GATGTGGCCATCACATTCGTCAGAT-3. The PCR cycling conditions consisted of initial denaturing step at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 30 s, then a final elongation step at 72°C for 7 min. The PCR products were separated on a 2% agarose gel and visualization with ethidium bromide. The *ACE* gene polymorphism was classified into three genotypes: the 190-bp deletion homozygous (DD) genotype, the 490-bp insertion homozygous (II) genotype, and the 490-bp insertion, 190-bp deletion heterozygous (ID) genotype.

Statistical analysis

Data were reported as means ± standard deviation (SD). The χ^2 test was used to evaluate categorical variables and test for Hardy–Weinberg equilibrium (HWE) of polymorphisms. Differences in biochemical parameters and BP were assessed using a paired *t*-test. Linear regression modelling was used to determine the correlation of BP response with genotypes, following normalization with pretreatment BP, age, gender, BMI, blood glucose, TC, TG, HDL and LDL. The SPSS 18 software was applied for statistical analysis. A two-tailed *P* value less than 0.05 was considered to be significant.

Table 1. Changes of patient characteristics and BP response to the treatment.

Characteristic	Pretreatment	Post-treatment	*P* value
BMI (kg/m^2)	25.8 ± 3.5	25.7 ± 3.3	0.562
SBP (mmHg)	159.5 ± 16.4	145.4 ± 20	< 0.001
DBP (mmHg)	101.5 ± 7.1	93.4 ± 10	< 0.001
HR (beats/min)	68.45 ± 10.85	68.23 ± 9.27	0.786
ALT (U/L)	24.33 ± 12.17	24.25 ± 12.73	0.938
AST (U/L)	25.98 ± 10.69	24.65 ± 10.92	0.340
Cr (mg/dL)	61.15 ± 12.42	60.01 ± 12.72	0.209
BUN (mmol/L)	5.55 ± 1.72	5.71 ± 1.78	0.314
UA (μ mol/L)	264.54 ± 83.79	256.43 ± 78.23	0.256
Glu (mmol/L)	5.44 ± 1.69	5.32 ± 1.65	0.398
TC (mmol/L)	4.67 ± 0.91	4.70 ± 0.91	0.636
TG (mmol/L)	1.67 ± 1.0	1.56 ± 1.2	0.165
LDL (mmol/L)	2.73 ± 0.78	2.74 ± 0.74	0.875
HDL (mmol/L)	1.34 ± 0.38	1.36 ± 0.38	0.260
Na^+ (mmol/L)	141.38 ± 2.96	140.99 ± 2.49	0.230
K^+ (mmol/L)	4.03 ± 0.42	4.10 ± 0.51	0.093
Cl^- (mmol/L)	102.98 ± 2.42	102.47 ± 2.46	0.068
Ang II (pg/mL)	76.3 ± 56.6	107.0 ± 66.7	< 0.001

Data are presented as mean ± SD. *P* value calculated by paired-samples T test. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Cr, creatinine; BUN, blood urea nitrogen; UA, uric acid; Glu, glucose; TC, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Na^+, sodium ion; K^+, potassium ion; Cl^-, chlorideion; AngII, angiotensin II.

Table 2. Genotype and allele frequencies of the *CYP11B2* –344C/T and *ACE* I/D gene polymorphisms in the study population.

Polymorphism	Genotype			Allele frequency	
CYP11B2 –344C/T	TT 76 (51.4)	TC 65 (43.9)	CC 7 (0.047%)	T 217 (73.3%)	C 79 (26.7%)
ACE I/D	II 58 (39.2%)	ID 72 (48.6%)	DD 18 (12.2%)	I 188 (63.5%)	D 108 (36.5%)

Results

Data from a total of 148 patients was analysed in this study. All patients had mild-to-moderate hypertension. The basic characteristics of patients are presented in table 1. After eight-week treatment with telmisartan, both SBP and DBP were remarkably decreased ($P < 0.001$). The serum angiotensin II level was obviously increased at the end of the 8-week treatment ($P < 0.001$). No significant differences were observed for the other parameters.

CYP11B2 –344C/T and ACE I/D gene polymorphism and antihypertensive response to telmisartan

Genotype and allele of *CYP11B2* –344C/T and *ACE* I/D gene, distributions of the study population are summarized in table 2. Genotype and allele frequencies of each polymorphism in the study population were in Hardy–Weinberg equilibrium ($P > 0.05$). The changes in BP response to antihypertensive treatment in relation to genotypes of *CYP11B2* –344C/T and *ACE* I/D gene polymorphisms are providedin table 3. Seven subjects carrying homozygous *CYP11B2* –344C allele were found, thus CC and CT genotypes were used in combination for analyses. We did not find any association of *ACE* I/D gene polymorphisms with SBP or DBP response to telmisartan. Surprisingly, linear regression analysis indicated that *CYP11B2* –344C/T was associated with DBP response to telmisartan, after correction with covariates, including pretreatment DBP, age, gender, BMI, blood glucose, TC, TG, HDL and LDL ($P = 0.005$). *CYP11B2* –344C/T accounted for 18.1% of inter-individual variation in the DBP response to telmisartan. The patients carrying the *CYP11B2* –344C allele (CC+CT) showed a greater reduction in DBP than those carrying the TT genotype (figure 1).

Discussion

Aldosterone is one of the main effectors of the RAAS. Aldosterone acts on the distal nephron to regulate sodium

Table 3. SNPs of *CYP11B2* –344C/T and *ACE* I/D gene in linear regression and BP response to antihypertensive drugs.

Genotype	Predictors in model	Systolic BP response			Diastolic BP response		
		Standardized β	P value	R^2	Standardized β	P value	R^2
CYP11B2 –344C/T	Alone	0.101	0.315	0.010	0.290	0.003	0.084
TT TC+CC	After covariates	0.217	0.354	0.093	0.298	0.005	0.181
ACE I/D II ID DD	Alone	0.124	0.219	0.015	0.011	0.912	0.010
	After covariates	0.212	0.567	0.056	0.012	0.908	0.098

SNPs were first respectively considered by themselves (alone) as a predictor of the BP response, then after adjustment for the concomitant variables including pretreatment BP, age, gender, BMI, glucose, TG, TC, HDL and LDL (after covariates). Standardized β is the standardized regression coefficient; R^2 is the percentage of inter individual variation in BP response explained by predictors in the model.

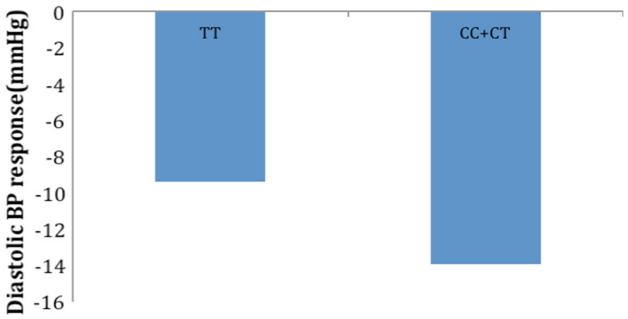

Figure 1. DBP response to telmisartan based on *CYP11B2* –344C/T genotypes after adjustment for pretreatment DBP, age, gender, BMI, glucose, TG, TC, HDL and LDL.

resorption, potassium excretion and intravascular volume. *CYP11B2* is the key rate-limiting enzyme in the final steps of aldosterone biosynthesis. A few polymorphisms in *CYP11B2* have recently been identified (White and Slutsker 1995; Fardella *et al.* 1996). Among them, –344C/T is a single-nucleotide polymorphism (SNP) located at a putative binding site for the transcription factor steroidogenic factor -1 (SF-1) (Fardella *et al.* 1996), 344 nucleotides from the transcription start site, where the residue could be a cytosine (–344C) or thymine (–344 T). This polymorphism has been associated with hypertension (Cheng and Xu 2009; Alvarez-Madrazo *et al.* 2013), coronary heart disease (Boduła *et al.* 2007) and has shown to influence aldosterone levels (Brand *et al.* 1998).

Several studies evaluated the impact of –344C/T polymorphism of *CYP11B2* on the response to antihypertensive drugs. One study on the association between the *CYP11B2*–344C/T gene polymorphism and antihypertensive response to hydrochlorothiazide (HCTZ) found that the BP response in patients with CC genotype was less obvious than that in others for male patients (Li *et al.* 2012). Another study indicated that the patients with TC and TT genotypes had significant reduction in BP after ACE inhibitor treatment (Yu *et al.* 2006). But the studies on the antihypertensive response to telmisartan were few. In our study, the reduction in DBP after eight weeks of telmisartan treatment was significantly greater in patients carrying the C allele (CC+CT) compared with that carrying the TT genotype ($P = 0.043$). We did not find an association between the –344C/T polymorphism and the reduction in SBP after treatment. ARBs, which inhibit the combination of angiotensin II and angiotensin II type 1 receptor, led to marked peripheral vasodilation and BP-lowering effects. Angiotensin II is a stimulus regulator of *CYP11B2*, which is the key rate-limiting enzyme in the final steps of aldosterone biosynthesis. Aldosterone controls sodium balance and intravascular volume and helps to regulate BP. It could be proposed that the C allele is associated with a higher expression of the *CYP11B2* gene and increased *CYP11B2* activity, which leads to higher aldosterone secretion and individuals with a variant in this gene could respond differently to ARBs.

The *ACE* I/D polymorphism is one of the more extensively studied genes in hypertension. This polymorphism consists of the insertion or deletion of some portion of a 287-bp sequence of nonsense DNA in intron 16 of the *ACE* gene. Some studies had investigated the relationship between the *ACE* I/D polymorphism and BP response to antihypertensive drugs. However, the results have been conflicting. A meta-analysis demonstrated that there was a significant association between *ACE* I/D polymorphism and BP responses to HCTZ (Choi *et al.* 2013). But the GENRES study reported that *ACE* I/D polymorphism does not markedly predict BP response to antihypertensive agents involved amlodipine, bisoprolol, HCTZ and losartan in white hypertensive men (Suonsyrjä *et al.* 2009). In this study, there were no significant differences in the reduction of SBP and DBP after treatment. However, there was also a tendency for a higher SBP reduction in patients carrying the DD genotype compared with those carrying the II or ID genotype after treatment.

In summary, the *CYP11B2* –344C/T polymorphism but not *ACE* I/D polymorphism was shown to influence the response to telmisartan in Chinese patients. Thus, specific genotypes might predict the response to specific antihypertensive treatment. This study has limitations because of

the relatively small size. Thus our study should be viewed as hypothesis-generating and should be followed by larger prospective studies to confirm the results.

Acknowledgement

Research supported by the Sino-German Laboratory for Molecular Medicine, FuWai Cardiovascular Hospital and Cardiovascular Institute, China.

References

Alvarez-Madrazo S., Mackenzie S. M., Davies E., Fraser R., Lee W. K., Brown M. *et al.* 2013 Common polymorphisms in the CYP11B1 and CYP11B2 genes: evidence for a digenic influence on hypertension. *Hypertension* **61**, 232–239.

Boduła A., Dołyk A., Protasiewicz J. and Adamiec R. 2007 The role of the –344C/T polymorphism of the aldosterone synthase gene (CYP11B2) in cardiovascular diseases. *Postepy Hig. Med. Dosw.* **61**, 838–847.

Brand E., Chatelain N., Mulatero P., Féry I., Curnow K., Jeunemaitre X. *et al.* 1998 Structural analysis and evaluation of the aldosterone synthase gene in hypertension. *Hypertension* **32**, 198–204.

Cheng X. and Xu G. 2009 Association between aldosterone synthase CYP11B2 polymorphism and essential hypertension in Chinese: a meta-analysis. *Kidney Blood Press. Res.* **32**, 128–140.

Choi H. D., Suh J. H., Lee J. Y., Bae S. K., Kang H. E., Lee M. G. *et al.* 2013 Effects of ACE and ADD1 gene polymorphisms on blood pressure response to hydrochlorothiazide: a meta-analysis. *Int. J. Clin. Pharmacol. Ther.* **51**, 718–724.

Do A. N., Irvin M. R., Lynch A. I., Claas S. A., Boerwinkle E., Davis B. R. *et al.* 2014 The effects of angiotensinogen gene polymorphisms on cardiovascular disease outcomes during antihypertensive treatment in the GenHAT study. *Front. Pharmacol.* **5**, 210.

Fardella C. E., Rodriguez H., Montero J., Zhang G., Vignolo P., Rojas A. *et al.* 1996 Genetic variation in P450c 11AS in Chilean patients with low rennin hypertension. *J. Clin. Endocrinol. Metab.* **81**, 4347–4351.

Gupta S., Chattopadhyaya I., Agrawal B. K., Sehajpal P. K. and Goel R. K. 2015 Correlation of renin angiotensin system (RAS) candidate gene polymorphisms with response to Ramipril in patients with essential hypertension. *J. Postgrad. Med.* **61**, 21–26.

Kim H. K., Lee H., Kwon J. T. and Kim H. J. 2015 A polymorphism in AGT and AGTR1 gene is associated with lead-related high blood pressure. *J. Renin Angiotensin Aldosterone Syst.* **16**, 712–719.

Kupari M., Hautanen A., Lankinen L., Koskinen P., Virolainen J., Nikkila H. *et al.* 1998 Associations between human aldosterone synthase (CYP11B2) gene polymorphisms and left ventricular size, mass, and function. *Circulation* **97**, 569–575.

Laragh J. H., Lamport B., Sealey J. and Alderman M. H. 1988 Diagnosis ex juvantibus. Individual response patterns to drugs reveal hypertension mechanisms and simplify treatment. *Hypertension* **12**, 223–226.

Li Y., Yang P., Wu S. L., Yuan J. X., Wu Y., Zhao D. D. *et al.* 2012 Effect of CYP11B2 gene -344 T/C polymorphism on renin-angiotensin-aldosterone system activity and blood pressure response to hydrochlorothiazide. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi.* **29**, 68–71.

Lind L., Berne C., Anderssno P. E., Hänni A. and Lithell H. 1995 Is insulin resistance a predictor of the blood pressure response to anti-hypertensive treatment? *J. Hum. Hypertens.* **9**, 759–763.

Liu D. X., Zhang Y. Q., Hu B., Zhang J. and Zhao Q. 2015 Association of AT1R polymorphism with hypertension risk: an update meta-analysis based on 28,952 subjects. *J. Renin Angiotensin Aldosterone Syst.* **16**, 898–909.

Materson B. J. 2004 Variability in response to antihypertensive drug treatment. *Hypertension* **43**, 1166–1167.

Patnaik M., Pati P., Swain S. N., Mohapatra M. K., Dwibedi B., Kar S. K. *et al.* 2014 Association of angiotensin-converting enzyme and angiotensin-converting enzyme-2 gene polymorphisms with essential hypertension in the population of Odisha, India. *Ann. Hum. Biol.* **41**, 145–152.

Preston R. A., Materson B. J., Reda D. J., Williams D. W., Hamburger R. J., Cushman W. C. *et al.* 1998 Age-race subgroup compared with rennin profile as predictors of blood pressure response to antihypertensive therapy. *JAMA* **280**, 1168–1172.

Suonsyrjä T., Hannila-Handelberg T., Fodstad H., Donner K., Kontula K. and Hiltunen T. P. 2009 Renin-angiotensin system and alpha-adducin gene polymorphisms and their relation to responses to antihypertensive drugs: results from the GENRES study. *Am. J. Hypertens.* **22**, 169–175.

Taverne K., de Groot M., de Boer A. and Klungel O. 2010 Genetic polymorphisms related to the renin-angiotensin-aldosterone system and response to antihypertensive drugs. *Expert Opin. Drug Metab. Toxicol.* **6**, 439–460.

Tiret L., Rigat B., Visviks S., Breda C., Corvol P., Cambien F. *et al.* 1992 Evidence from combined segregation and linkage analysis that a variant of the ACE gene controls plasma levels. *Am. J. Hum. Genet.* **1**, 197–205.

Venter J. C., Adams M. D., Myers E. W., Li P. W., Mural R. J., Sutton G. G. *et al.* 2001 The sequence of the human genome. *Science* **291**, 1304–1351.

White P. C. and Slutsker L. 1995 Haplotype analysis of CYP11B2. *Endocr. Res.* **21**, 437–442.

Yu H. M., Lin S. G., Liu G. Z., Zhang Y. Q., Ma W. J. and Deng C. Y. 2006 Associations between CYP11B2 gene polymorphisms and the response to angiotensin-converting enzyme inhibitors. *Clin. Pharmacol. Ther.* **79**, 581–589.

Corresponding editor: H. A. Ranganath