Angiotensin II type 2 receptor gene polymorphisms and essential hypertension[1]

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KEY WORDS angiotensin II; angiotensin receptors; hypertension; polymorphism (genetics); single nucleotide polymorphism

ABSTRACT

AIM: To identify the genetic variants of angiotensin II type 2 receptor (AT_2R) gene in a Chinese population and to determine whether the AT_2R gene polymorphisms are associated with essential hypertension (EH). **METHODS:** The detection of single nucleotide polymorphisms (SNPs) was performed in 19 subjects by a direct DNA sequencing. Two hundred fifty patients with EH and 250 nomortensive controls were included in the study to assess the contribution of polymorphism of AT_2R gene to hypertension. **RESULTS:** We identified 9 SNPs in the promoter, intron, exons and 3' untranslated region (3'UTR) of AT_2R gene; among them 5 SNPs were novel molecular variants. A case-control study using a most frequent SNP (1334T/C) in the promoter region, showed a significant increase in allele frequency of C^{1334} in male hypertensive subjects (17.5 % *vs* 10.3 % for normotensive subjects, $P<0.05$). **CONCLUSION:** The catalogue of SNPs of AT_2R gene in Chinese population showed ethnic difference in DNA sequence variation. A polymorphism in the promoter region (1334T/C) of AT_2R gene might be involved in the development of hypertension in Chinese population.

INTRODUCTION

Hypertension is a major risk factor for stoke, myocardial infarction, congestive heart failure, and end stage renal disease[1,2]. The renin-angiotensin system (RAS) plays a key role in salt and water homeostasis and vascular tone regulation. It is well accepted that angiotensin II (Ang II), the central product of the system, causes its effects via at least two major receptor subtypes, Ang II typer 1 receptor (AT_1R) and type 2 receptor (AT_2R)[3]. The AT_1R mediates almost all the known effects of Ang II in human, such as vasoconstriction, release of aldosterone, renal sodium absorption, vascular cell growth and so on[4]. In contrast to many data on the role of AT_1R, the role of AT_2R in vascular diseases remains to be defined. Although the relationships between the polymorphisms of RAS genes and hypertension have been extensively studied, there are few reports of the association between the human AT_2R gene and essential hypertension (EH)[5].

The aim of the present study was to screen for the genetic variants in AT_2R gene in a Chinese popula-

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tion and to assess the association between the polymorphisms of AT_2R gene and EH through a case-control study.

MATERIALS AND METHODS

Subjects All subjects in this study are of Han Chinese currently residing in Shanghai area. DNA samples from 19 unrelated hypertensive subjects were used for detection of single nucleotide polymorphism (SNP). Two hundred and fifty unrelated patients with EH and 250 normotensive (NT) subjects were enrolled for association study. The inclusion criteria for the cases were as follows: (1) at least three consecutive blood pressure measurements ≥140 mmHg for systolic blood pressure (SBP) and/or ≥90 mmHg for diastolic blood pressure (DBP), or currently receiving antihypertensive drugs for at least 1 year, (2) an onset of hypertension after 30 and before 60 years ago, (3) no clinical or biochemical signs of secondary hypertension. The subjects recruited for the controls had the following characteristics: (1) SBP<140 mmHg and DBP<90 mmHg, (2) age at the time of the study ≥40 years.

Blood pressure was measured in the seated position after 10 min of rest using a mercury sphygmomanometer by experienced and certified examiners. Afterwards, a detailed questionnaire was filled out, a physical examination was carried-out and 10 mL blood sample was drawn from all subjects for DNA extraction and biochemical examination. Informed consent was obtained from all participants.

SNP detection and genotyping Genomic DNA was extracted from peripheral white cells using a standard phenol-chloroform method.

The detection of SNPs was performed by a direct DNA sequencing. After PCR amplification and purification, all the exons, flanking introns and the promoter region of AT_2R gene were sequenced. The primers were designed with Primer 3 software developed at Whitehead Institute and Howard Hughes Medical Institute (http://www-genome.wi.mit.edu/genome_software/other/primer3.html). In brief, "touchdown PCR"[6] was performed under standard conditions in a total volume of 10 μL on GeneAmp 9700 PCR System (Applied Biosystems). DNA was denatured once for 2 min at 94 ºC, following 40 cycles of denaturing (94 ºC, 30 s), annealing (58 ºC, 40 s) and extension (72 ºC, 40 s), except that in the first 10 cycles, the annealing temperatures decreased from 63 ºC to 58 ºC by 0.5 ºC a cycle. One final extension step for 7 min at 72 ºC was added. Wizard PCR Preps DNA Purification Resin (Promega) was used for purification. The PCR products were analyzed on ABI-PRISM 377 Automatic sequencer (Applied Biosystems). SNPs were detected with Polyphred Program that developed at University of Washington (http://droog.mbt.washington.edu/Polyphred.html).

Genotypes of the SNP03 in the AT_2R promoter region were determined by PCR amplification followed by direct DNA sequencing as above-mentioned.

Statistical analysis Results were presented as mean±SD. Differences in the distribution of genotypes between groups were determined by the χ^2 test. $P<0.05$ was considered statistically significant.

RESULTS

Identification of AT_2R variants Nineteen hypertensive subjects were enrolled in the initial search for AT_2R gene variants. In a total length of 2938 kb sequenced, 9 SNPs were identified, of which 3 in the promoter region, 1 in intron, 2 in exons, and 3 in 3' untranslated region (3'UTR), respectively. Tab 1 shows the position and base substitutions of these SNPs.

Among them, 4 SNPs (SNP 04,06,07,08) were listed in the database of SNPs of the National Center of Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) and the other 5 SNPs (SNP01,02,03,05,09) were detected for the first time in the Chinese population. The frequency of each allele was calculated and shown in Tab 2. The alleles of 2 SNPs (SNP01, 02) in promoter region and 2 SNPs (SNP05, 06) in exons were with lower frequency (<10 %).

Association between hypertension and AT_2R polymorphisms The genotype for a most frequent variant (SNP03, 1334T/C) located in promoter region was then performed in 250 EH and 250 NT subjects. Clinical characteristics from both groups were presented in Tab 3. The C^{1334} was significantly more frequent in the male EH group compared with the male NT group (17.5 % *vs* 10.3 %, $P<0.05$, Tab 4).

Values are means±SD. EH, essential hypertension; NT, normotensive control; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FG, Fasting glucose; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoproteins; HDL, high-density lipoproteins. BUN, blood urea nitrogen; Cr, Creatinine; and UA, uric acid.

Tab 1. SNPs detected within AT_2R gene.

SNP name	NCBI SNP cluster ID	Position of AT_2R gene	Location in gene	Base substitution and flanking Sequence
SNP01	new	362	promoter	ttcctgattt**G/A**taaagtgggg
SNP02	new	438	promoter	actttaaaca**T/C**attagctcat
SNP03	new	1334	promoter	ttggagacag**T/C**gagaatttca
SNP04	rs1403543	1675	intron	caaaactcct**A/G**aattatttag
SNP05	new	3066	exon	gtcttcactt**C/T**gggcttgtga
SNP06	rs3729977	3697	exon	agcaacatgc**T/C**attttggaat
SNP07	rs5193	4297	3'UTR	gtacaagatt**G/T**tcattggtga
SNP08	rs5194	4303	3'UTR	gattgtcatt**G/A**gtgagacata
SNP09	new	4599	3'UTR	tctttaaaaa**C/A**gctataaatt

Tab 2. Allele frequencies of SNPs of AT_2R gene.

SNP name	Allele1	Allele2	Frequency of allele1		Frequency of allele2	
			Male	Female	Male	Female
SNP01	G	A	1	0.98	0	0.02
SNP02	T	C	1	0.98	0	0.02
SNP03	T	C	0.85	0.83	0.15	0.17
SNP04	A	G	0.7	0.58	0.3	0.42
SNP05	C	T	1	0.96	0	0.04
SNP06	T	C	1	0.98	0	0.02
SNP07	G	T	0.9	0.87	0.1	0.13
SNP08	G	A	0.7	0.58	0.3	0.42
SNP09	C	A	0.7	0.58	0.3	0.42

DISCUSSION

AT_2R is highly expressed in fetal life and declines rapidly after birth[7,8]. AT_2R expression is restricted to a few tissues in adults. In the adult, AT_2R mRNA has been detected in the adrenal gland, heart, brain, and its protein has been detected in the kidney[7,9,10]. During the past few years, studies have demonstrated that the AT_2R is responsible for vasodilation, natriuresis, and antiproliferation thus AT_2R appears to play a counter regulatory protective role opposing the AT_1R[11-19]. Recently, animal model studies have indicated that AT_2R plays an important role in the regulation of blood pressure[20,21].

The human AT_2R gene about 5 kb in length is lo-

Tab 3. Characteristics of hypertensive and normotensive subjects.

Parameter	Total			Male			Female		
	EH (*n*=250)	NT (*n*=250)	*P*	EH (*n*=137)	NT (*n*=126)	*P*	EH (*n*=113)	NT (*n*=124)	*P*
Age (years)	57.03±12.09	49.94±5.47	<0.01	56.92±12.79	51.57±5.02	<0.01	57.16±11.25	48.27±5.44	<0.01
BMI (kg/m^2)	25.66±12.09	23.99±2.69	<0.01	25.45±4.00	23.28±2.47	<0.01	25.92±3.78	22.70±2.88	<0.01
SBP (mmHg)	156±18	113±10	<0.01	155±17	114±10	<0.01	156±20	111±11	<0.01
DBP (mmHg)	97±12	74±8	<0.01	98±12	75±7	<0.01	96±12	72±8	<0.01
FG (mmol/L)	5.66±1.13	4.77±0.53	<0.01	5.62±1.03	4.70±0.55	<0.01	5.72±1.26	4.83±0.51	<0.01
TC (mmol/L)	4.81±0.99	4.46±0.73	<0.01	4.72±1.00	4.52±0.71	0.071	4.93±0.97	4.39±0.76	<0.01
HDL (mmol/L)	1.16±0.31	1.43±0.34	<0.01	1.12±0.30	1.33±0.31	<0.01	1.20±0.32	1.55±0.34	<0.01
TG (mmol/L)	2.00±1.02	1.24±0.62	<0.01	2.09±1.17	1.39±0.62	<0.01	1.89±0.78	1.09±0.58	<0.01
LDL (mmol/L)	2.81±0.83	2.77±0.77	0.576	2.74±0.83	2.97±0.76	<0.05	2.90±0.82	2.51±0.72	<0.01
BUN (mmol/L)	6.23±1.99	5.28±1.28	<0.01	6.71±2.33	5.54±1.38	<0.01	5.65±1.28	5.02±1.12	<0.01
Cr (μmol/L)	85±35	84±14	0.779	99±40	92±12	0.061	76±22	89±36	<0.01
UA (μmol/L)	357±94	292±82	<0.01	389±97	339±70	<0.01	319±74	244±62	<0.01

Tab 4. Comparison of genotype and allele frequency for variant 1334T/C in hypertensive and normotensive subjects.

			Genotypes			Allele frequency			
			TT	TC	CC	T	C	$\chi 2$	*P*
Male	EH	137	113	0	24	0.825	0.175	5.63	<0.05
	NT	126	113	0	13	0.897	0.103		
Female	EH	113	72	39	2	0.810	0.190	1.732	0.188
	NT	124	93	26	5	0.854	0.145		

EH, essential hypertension; NT, normotensive control.

cated on X chromosome, it includes 3 exons and regulatory elements in the first intron and promotor region[22-24]. According to the SNPs data of NCBI, there are 15 SNPs in human AT_2R gene. However, only 2 intron SNPs were listed in Japanese nucleotide polymorphisms database (http://snp.ims.u-tokyo.ac.jp/). It suggests that important ethnic difference in SNPs distribution might exist. Therefore, it is necessary to establish the database of SNPs in Chinese nation. In this study, we first identified the molecular variants within AT_2R gene using direct DNA sequencing, the most solid technique for mutation detection. Nine SNPs were detected in Chinese, among them 4 SNP were listed in the NCBI database, and the other 5 SNPs were detected for the first time within AT_2R gene.

We selected SNP03 (1334T/C) to test its potential association with hypertension for the following reasons (1) 3 SNPs (SNP03 in promoter region, SNP04 in intron and SNP05 in exon) were previously tested in a pilot association study including 96 hypertensive and 96 normotensive subjects. Preliminary results showed a positive association of SNP03 with hypertension (data not shown); (2) SNP03 is a most frequent variant located in the promoter region. The T to C substitution will result in the lost of a transcriptional binding site.

It was worthwhile to note that 3 transcriptional binding sites, V$COMP1.01, V$RBPJK.01, and V$HMGIY.01 were involved in this polymorphism location (http://www.genomatix.de). V$RBPJK.01, the binding site for mammalian transcriptional repressor RBP-JKappa/CBF1, would be lost when the T to C polymorphism happened. It suggests that this SNP might be related to the expression of AT_2R gene, and further evidence need to substantiate the functional significance concerned.

We observed a positive association between AT_2R 1334T/C polymorphism and hypertension only in male. A possible explanation is that the AT_2R gene is located on X chromosome, therefore it might exert differential influence on male and female. Nevertheless, the exact mechanisms remain to be evaluated.

In conclusion, this is the first study to catalog the SNPs of AT_2R gene in Chinese and a polymorphism in the promoter region of the human AT_2R gene was associated with EH in male Chinese. This results need to be verified in another large population and the functional significance of the SNP deserves to be further explored.

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