The polymorphism of angiotensin-receptor gene A1166C in familial hypertension and its distribution in the Han Yellow race of China

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ABSTRACT

الأهداف: دراسة تعدد أشكال مستقبل جين أنجيوتنسين وأليل جين A1166C في مرض ضغط الدم الوراثي وانتشاره في سلالة هان الصفراء في الصين.

الطريقة: اشتملت الدراسة على 104 مريض بارتفاع ضغط الدم ليس لديهم أي تاريخ وراثي في العائلة و 178 مريض بتاريخ وراثي و 150 شخص تم اختيارهم بطريقة عشوائية وضمهم في هذه الدراسة المستقبلية واكتشاف الجيني بطريقة تفاعل السلسلة المبلمرة و طول تعدد الأشكال في التجارب. أجريت الدراسة خلال الفترة من اكتوبر 2009م حتى نوفمبر 2010م في مركز جينان، الأمراض الوعائية، المستشفى التابع لكلية تيشان، جينان، الصين.

النتائج: ظهرت نتائج إحصائية مهمة في تعداد الجين وأليل جين A1166C في مجموعة الضغط الوراثية,(x^2=7.663 p=0.020) بالمقارنة مع مجموعة الشاهد الطبيعية,(x^2= 8.288 p=0.004). كما أن هذه النتائج لم تكن مختلفة إحصائياً بين مجموعة الضغط الوراثية (x^2=1.289, p=0.322) ومجموعة الشاهد الطبيعية (x^2=1.289, p=0.256) في تعداد الجين وأليل جين A1166C . كما لم تظهر أي اختلافات إحصائية بين المجموعات الثلاث بين العمر، والجنس في توزيع الجين والأليل جين A1166C (p>0.05) .

خاتمة: أن انتشار النمط الجيني وأليل جين A1166C لدى المرضى مع التاريخ العائلي للمرض بشكل أكثر من الأشخاص الأصحاء وذلك في مجتمع سلالة هان الصفراء في الصين. كما أن انتشار مستقبل جين أنجيوتنسين ليس له علاقة بالعمر والجنس.

Objectives: To study the polymorphism of angiotensin-type 1 receptor (AT1R) gene A1166C in familial primary hypertension and its distribution in Han Yellow race of China.

Methods: One hundred and four hypertensive patients with no family history, 178 hypertensive patients with familial history, and 150 healthy adults were randomly selected to participate in a prospective clinical trial, and genetype detection by standard polymerase chain reaction methods and restriction fragment length polymorphism (PCR-RFLP) in trials. The study was carried out between October 2009 and November 2010 at Jinan Center for Cardiovascular Disease, Affiliated Hospital of Taishan Medical College, Jinan, China.

Results: Notable statistical significances exist in the frequency of genotype and allele of A1166C in familial hypertension group (x^2=7.663, p=0.020) compared with the normal control group (x^2= 8.288, p=0.004). No significant difference was found in the hypertension group (x^2 = 2.186, p=0.322) compared with the normal control group (x^2=1.289, p=0.256) in the frequency of genotype and allele of A1166C. No significant differences were found between various ages or genders in each of the 3 groups in genotype and allele of A1166C (p>0.05) distribution.

Conclusion: In the Han Yellow race population of China, the frequency of genotype and allele of A1166C of patients with familial hypertension is higher than that of healthy adults. The distribution of AT1R gene polymorphisms of A1166C is not related to age or gender.

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Few studies have examined in the literature about the gene distribution characteristics in the normal population in Han Yellow race of China at present. For the past 2 decades, genetic variants of the RAS (renin-angiotensin system)have been tested for association with blood pressure response, but the results have been inconsistent.[1] We choose the same area, the same nationality, genetic gathered of high rate of familial hypertension patients as the research object, examining the association with AT1R A1166C gene polymorphism, and observing the distribution of AT1R gene A1166C polymorphism in the Han Yellow race people of China. The aim of our study was to provide evidence for the etiology of hypertension. The study was carried between October 2009 and November 2010 at Jinan Center for Cardiovascular Disease, Affiliated Hospital of Taishan Medical College.

Methods. It was a prospective clinical trial. Participates were randomly selected and randomly numbered. All of them are Han Yellow race people, native of Jinan City, Shandong Province, long-term residents (more than 5 years). Hospital Ethics Committee had approved. All participants had signed informed consent. All selected pump peripheral venous blood lipids, glucose, and DNA (Deoxyribonucleic Acid)was extracted from peripheral blood on an empty stomach early in the morning, meanwhile measure weight, height. With blood collection in a quiet environment using the Shanghai of Yutu brand Tyrode mercury sphygmomanometer the right the upper extremity seat brachial artery blood pressure. Three hours before the measurement is not drinking, not smoking, blood pressure measurement in the sit-10min 3 times, 3 times the difference in blood pressure ≤4 mmHg (1 mm Hg 0.133kpa), the mean value. Hypertension inclusion criteria: According to 2003 WHO/ISH[2] (the World Health Organization/International Society of Hypertension)hypertension diagnostic criteria that SBP (systolic blood pressure) ≥140 mm Hg and (or) DBP (diastolic blood pressure) ≥90 mm Hg or previous diagnosis of hypertension (in accordance with the above criteria) are receiving drug treatment. Family history defined as parents or siblings at least one suffering from primary hypertension. We excluded various cardiovascular and cerebrovascular diseases, diabetes and liver, kidney, thyroid disease history. One hundred fifty cases of healthy adults as a control group, 93 cases were male and 57 females, mean age (68.0±7.7) years old. Primary (non-familial) hypertensive patients with 104 cases of primary hypertension group, including 55 males and 49 females, mean age (65.0±5.7) years. The 178 cases of patients with familial hypertension for the familial hypertension group, 103 cases were male, female 75 cases, the average age (65.2±6.5) years of age, ranging from 10-20 years of history of hypertension, an average of 10.5 years (**Table 1**).

Deoxyribonucleic acid extraction. We extracted the whole blood DNA using the hypotonic hemolysis-salting. Peripheral venous blood 2ml, eathylene diamine tetraacetic acid (EDTA) anticoagulant, store at -20°C, batch extraction. Leukocyte lotion 2ml washed repeatedly and centrifuged 3 times to precipitate. Coupled with protein cell lysate 2ml, protease K 250μg (end to a concentration of 0.1g/L). Digested overnight at 37°C constant temperature water bath, adding 1 ml saturated brine, oscillation centrifugation, rinse with ethanol and centrifuged again, dried precipitate. The UV spectrophotometer absorbance (A) values. The purity of A260: A280 reach 1.8.

Polymerase chain reaction (PCR) amplification of the objective DNA fragment. The A1166C gene polymorphisms of AT1R were determined with the use of standard polymerase chain reaction methods and restriction fragment length polymorphism (PCR-RFLP). Polymerase chain reaction primers (Shanghai SANGON biological engineering company, China), primer 1: 5`-GAG ATT GCA TTT CTG TCA GT-3`; primer 2: 5`-ATA ATG TAA GCT CAT CCA CC-3`; Primers per tube are 0.24pmol/L, dNTP 0.1 mmol/L concentration of Mg2 +2.0mmol/L, TagDNA polymerase (Takara Biotechnology Co. Ltd, Dalian, China) 2U, 10 × buffer 5μl, template DNA 0.2 μg. Add sterile double distilled water to make a volume of 50 μL, blending, instantaneous cryogenic centrifugal. Cycle response in DNA thermal cycler (PTC-100, Minicycler M J Research, United States of America) on the following conditions: 94°C preliminary denaturation for 4 minutes, 94 denaturation 1 minute, 62°C annealing 1 minute, 72°C extending 1.5 minutes, complete 30 cycles, 72 continue extending for 5 minutes. Agarose gel containing ethidium bromide electrophoresed for 30 minutes in the voltage 100V, 1 × TAE (Tris-acetate-EDTA) buffer. Ultraviolet light observed amplification results.

Polymerase chain reaction product purification and enzyme digestion. The PCR product was purified by saturated phenol, chloroform/isoamyl alcohol, ethanol, etc. Purified PCR amplification products 8μl, 5U Dde I endonuclease (The United States of America Promega company) 10ENB buffer 2μl, plus double distilled water to make volume 20μl, 37°C warm bath for 4.5 hours. Agarose gel containing ethidium bromide electrophoresed for 45 minutes in the voltage 100V, 1

× TAE buffer. Observed gel imaging system and save digested results.

Statistical analysis. Data analyses were performed with the SPSS Version 17, all measurement data using the mean ± standard deviation, compared using t test; genetic equilibrium test using the Hardy-Weinberg equilibrium; genotype and gene frequency comparison with the χ^2 test. Differences between the groups of the allele frequencies and genotype distributions were analyzed by Fisher's exact test when necessary. The significance level for statistical test was 0.05(p<0.05).

Results. The gender, age, and other clinical indicators of the 3 groups were comparable (p>0.05). The essential hypertension and familial hypertension group, systolic blood pressure (SBP) and diastolic blood pressure (DBP) was significantly higher than the normal control group (p<0.05); gender, age, body mass index, fasting blood glucose, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), no significant difference existed between the 2 groups (p>0.05).

AT1R gene A1166C polymorphism analysis. Successfully amplified DNA samples of 432 cases. The PCR amplification product is the 350bp fragment, Dde sites of the restriction edonuclease (C▲TNAG) is generated if the amplified fragment in the presence of the A1166-C nucleotide replaces. If the digested generate a 350bp fragment, it is a homozygous, genotype is AA. If 2 fragments of 210bp and 140 bp, it is the homozygous mutation, genotype is CC; If there is 350bp, 210bp, 140bp3 fragments it is heterozygous, genotype is AC (**Figure 1**). Normal control group AT1R 1166 locus genotype distribution frequency observations AA, AC, CC were 129 cases, 19 cases, 2 cases, according to the principles of Hardy-Weinberg equilibrium, AA, AC, CC expected values were 128 cases, 20 cases, 2 cases (χ^2 = 0.03, p>0.05), satisfied the Hardy-Weinberg equilibrium.

AT1R gene A1166C polymorphism of the familial hypertensive patients. The essential hypertension genotype distribution were AA 83 (79.8%), AC 20 (19.2%), CC 1 (1.0%), allele frequencies were A186 (89.4%), C 22 (10.6%). Familial hypertension group genotype distribution were AA 131 (73.6%), AC41 (23.0%), CC6 (3.4%), allele frequencies were A 303 (85.1%), C 53 (14.9%). The 2 sets of genotype distribution was no significant difference in the frequency and predictive value, accord with Hardy-Weinberg equilibrium. Compared with the normal control group, the genotype and allele frequency distribution of essential hypertension group, no significant difference existed (χ^2 = 2.186, p=0.322; χ^2 = 1.289, p=0.256, **Table 2**). Familial hypertension group, genotype and allele frequency distribution, compared with the normal control group, a significant difference

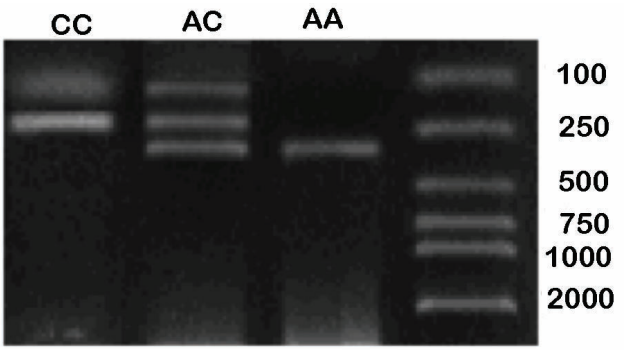

Figure 1 - The digested results electrophoresis.

Table 1 - Comparison of the main predicate interchange of the 3 groups of general information.

Groups cases	Normal control group (N=150)	Essential hypertension group (n=104)	Familial hypertension group (n=178)
Gender (male/female)	93/57	55/49	103/75
Age (years)	68.0±7.7	65.0±5.7	65.2±6.5
BMI (kg/m^2)	26.11±2.14	24.30±2.90	26.31±2.63
Fasting glucose(mmol/L)	5.29±0.82	4.91±1.02	5.45±0.95
TC (mmol/L)	5.19±0.96	5.11±1.06	5.17±1.02
TG (mmol/L)	1.66±0.62	1.43±1.41	1.62±0.58
HDL (mmol/L)	1.09±0.37	1.08±0.36	1.07±0.33
SBP (mm Hg)	127.60±11.54	160.23±19.02*	162.23±17.02*
DBP (mm Hg)	77.81±6.32	105.72±12.75*	103.72±10.75*

Compared with the normal control group, *p<0.05.
BMI - body mass index, TC- total cholesterol, TG - triglycerides, HDL - high density lipoprotein, SBP - systolic blood pressure, DBP - diastolic blood pressure

Table 2 - Primary, familial hypertension group, and normal control group genotype.

Groups	Genotypic			A allele	C allele
	AA	AC	CC	frequency	frequency
Normal control group	129 (86.0)	19 (12.7)	2 (1.3)	277 (92.3)	23 (7.7)
Essential hypertension group	83 (79.8)	20 (19.2)	1 (1.0)	186 (89.4)	22 (10.6)
Familial hypertension group	131(73.6)	41 (23.0)	6 (3.4)	303 (85.1)	53 (14.9)
Data are expressed as number and percentage (%)					

Table 3 - Genotype and gene frequencies of different gender and age in the normal control group.

Groups cases	The genotypes (example)			Allele frequency	
	AA	AC	CC	A	C
				n (%)	n (%)
Male (n=93)	81	10	2	172 (92.5)	14 (7.5)
Female (n=57)	48	9	0	105 (92.1)	9 (7.9)
<40 years (n=57)	51	6	0	108 (94.7)	6 (5.3)
40~60 years (n=69)	58	10	1	126 (91.3)	12 (8.7)
>60 years (n=24)	20	3	1	43 (89.6)	5 (10.4)

Table 4 - Genotype and gene frequencies of different gender and age in the essential hypertension group.

Groups cases	Genotypes			Allele frequency	
	AA	AC	CC	A	C
	n	n	n	n (%)	n (%)
Male (n=55)	44	11	0	99 (90.0)	11 (10.0)
Female (n=49)	39	9	1	87 (88.8)	11 (11.2)
<40 years (n=40)	33	7	0	73 (91.3)	7 (8.8)
40-60 years (n=49)	38	10	1	86 (87.8)	12 (12.2)
>60 years (n=15)	12	3	0	27 (90.0)	3 (10.0)

Table 5 - Genotype and gene frequencies of different gender and age in the familial hypertensional group.

Groups cases	Genotypes			Allele frequency	
	AA	AC	CC	A	C
				n (%)	n (%)
Male (n=103)	76	22	5	174 (84.5)	32 (15.5)
Female (n=75)	55	19	1	129 (86.0)	21 (14.0)
<40 years (n=66)	52	12	2	116 (87.9)	16 (12.1)
40-60 years (n=84)	60	22	2	142 (84.5)	26 (15.5)
>60 years (n=28)	19	7	2	45 (80.4)	11 (19.6)

existed (χ^2 = 7.663, p=0.020; χ^2 = 8.288, p=0.004. **Table 2**).

The distribution characteristics of AT1R gene A1166C polymorphism. We observed that there were no differences between the various age or gender in each of the 3 groups. In the normal control group, AA, AC and CC genotypes and the point A, C allele frequencies, compared between the different gender (χ^2 = 1.627, p=0.413; χ^2 = 0.014, p=0.907) (**Table 3**) and age (Fisher's exact test: χ^2 = 2.884, p=0.601; χ^2 = 1.109, p=0.292) (**Table 3**); In essential hypertension group, AA, AC and CC genotypes and the point A, C allele frequencies, compared between the different sex (χ^2 = 1.113, p=0.798; χ^2 = 0.082, p=0.774) (**Table 4**) and age (χ^2 = 1.748, p=0.973; χ^2 = 0.581, p=0.748) (**Table 4**). In the familial hypertension group, AA, AC and CC genotypes and the point A, C allele frequencies, compared between the different sex (χ^2 = 1.726, p=0.416; χ^2 = 0.161, p=0.688) (**Table 5**) and age (χ^2 = 3.221, p=0.503; χ^2 = 1.843, p=0.398) (**Table 5**).

Discussion. Angiotensin (Ang) receptor exists type 1 and type 2 receptor. Angiotensin II most physiological functions, such as vasomotor water-salt metabolism, myocardial fibrosis and vascular wall, the release of endothelin guide are mediated primarily

through AT1R. Genetic factors determined coding and non-coding regions of AT1R, leading to different shear, thus affecting the occurrence of cardiovascular disease. Coding and non-coding regions of AT1R at least have 5 mutation sites (T573C, A1062G, A1166C G1517T, and A1878G). AT1R gene A1166C polymorphism associated with a variety of cardiovascular diseases or factors, such as hypertension, atherosclerosis, myocardial infarction, coronary vasomotor movement, left ventricular mass, its pathogenic mechanism is unclear.

There is no unanimous conclusion on the study of AT1R gene A1166C polymorphism with primary hypertension. Agachan study the Turkish population to found that the C1166 allele frequency was significantly higher in the group with hypertension.[3] The majority of the reason think that a functional nucleotide sequence of the AT1R gene linkage disequilibrium, affecting gene to regulate angiotensin II. However, there are studies that AT1R gene A1166C polymorphism and essential hypertension unrelated. Gardier thinks that AT1R gene A1166C polymorphism has something to do with hypertension atherosclerosis, and has nothing to do with high blood pressure.[4] Freitas's[5] research on Brazil's rural population and Croatian scholars Barbalić M's[6] research did not draw firm conclusions. As a major component of RAS, the AT1 variant is one of the most pharmacogenomic candidates for blockade intervention of the system. Recent studies have consistently found no difference for A1166C in blood pressure reduction by ARBs (angiotensin II receptor blockers),[7-9] and ACE (angiotensin-converting enzyme) inhibitors.[10-12] We may conclude that the conventional AT1 gene variant, A1166C, is not associated with the antihypertensive effects of RAS blockade. But, very recently, Chinese scholars, Zhang et al[13] determined that the AT1R A1166C polymorphism may increase the antihypertensive effect of benazepril in patients with hypertension using the Family-Based Association Test (FBAT).

Population distribution characteristics of AT1R gene A1166C in normal people rarely reported in current literature in China. Hospitalized patients often suffer from variety, complex diseases at the same time. They have different risk factors, lifestyle and living environment. There are a lot of differences compared with community population. It will lead more Selection bias if choosing hospitalized patients as the object of study. We select the same region, the nation, the living environment and eating habits similar, genetic aggregation rate of patients with familial hypertension in study, reducing the probability of false positive in the test. When we collect specimen, we try to keep comparable between the 2 groups of age, gender, body mass index, and other factors. The study found that the frequency of -1166C allele increased in the familial hypertension and there was no significant difference between the different sex and age in Han Yellow race. The study provides a new basis for the relation of familial hypertension and polymorphism of A1166C in Chinese Han Yellow race population. Due to the lack of funds and the objective conditions, this study has limitations. This is a single gene association study, the size of the sample is relatively low. It needs larger samples or linkage analysis for a further research.

References

1. Konoshita T; Genomic Disease Outcome Consortium (G-DOC) Study Investigators. Do genetic variants of the Renin-Angiotensin system predict blood pressure response to Renin-Angiotensin system-blocking drugs?: a systematic review of pharmacogenomics in the Renin-Angiotensin system. ***Curr Hypertens Rep*** 2011; 13: 356-361.
2. 2003 World Health Organization(WHO)/International Society of Hypertension (ISH). Statement on management of hypertension. ***J Hypertens*** 2003; 21: 432.
3. Agachan B, Isbir T, Yilmaz H, Akoglu E. Angiotensin converting enzyme I/D, angiotensinogen T174M-M235T and angiotensin II type 1 receptor A1166C gene polymorphisms in Turkish hypertensive patients. ***Exp Mol Med*** 2003; 35: 545-549.
4. Gardier S, Vincent M, Lantelme P, Rial MO, Bricca G, Milon H. A1166C polymorphism of angiotensin II type 1 receptor, blood pressure and arterial stiffness in hypertension. ***J Hypertens*** 2004; 22: 2135-2142.
5. Freitas SR, Cabello PH, Moura-Neto RS, Dolinsky LC, Bóia MN. Combined analysis of genetic and environmental factors on essential hypertension in a brazilian rural population in the Amazon region. ***Arq Bras Cardiol*** 2007; 88: 447-451.
6. Barbalić M, Skarić-Jurić T, Cambien F, Barbaux S, Poirier O, Turek S, et al. Gene polymorphisms of the renin-angiotensin system and early development of hypertension. ***Am J Hypertens*** 2006; 19: 837-842.
7. Nordestgaard BG, Kontula K, Benn M. Effect of ACE insertion/deletion and 12 other polymorphisms on clinical outcomes and response to treatment in the LIFE study. ***Pharmacogenet Genomics*** 2010; 20: 77-85.
8. Konoshita T, Kato N, Fuchs S, Mizuno S, Aoyama C, Motomura M, et al. Genetic variant of the Renin-Angiotensin system and diabetes influences blood pressure response to Angiotensin receptor blockers. ***Diabetes Care*** 2009; 32: 1485-1490.
9. Redon J, Luque-Otero M, Martell N, Chaves FJ; POLPRI Investigators. Renin-angiotensin system gene polymorphisms: relationship with blood pressure and microalbuminuria in telmisartan-treated hypertensive patients. ***Pharmacogenomics J*** 2005; 5: 14-20.

10. Filigheddu F, Argiolas G, Bulla E, Troffa C, Bulla P, Fadda S, et al. Clinical variables, not RAAS polymorphisms, predict blood pressure response to ACE inhibitors in Sardinians. ***Pharmacogenomics*** 2008; 9: 1419-1427.
11. Yu H, Lin S, Jin L, Yu Y, Zhong J, Zhang Y, Liu G. Adenine/cytosine(1166) polymorphism of the angiotensin II type 1 receptor gene and the antihypertensive response to angiotensin-converting enzyme inhibitors. ***J Hypertens*** 2009; 27: 2278-2282.
12. Brunner M, Cooper-DeHoff RM, Gong Y, Karnes JH, Langaee TY, Pepine CJ, et al. Factors influencing blood pressure response to trandolapril add-on therapy in patients taking verapamil SR (from the International Verapamil SR/Trandolapril [INVEST] Study). ***Am J Cardiol*** 2007; 99: 1549-1554.
13. Zhang N, Cui H, Yang L. Effect of angiotensin II type I receptor A1166C polymorphism on benazepril action in hypertensive patients: a family-based association test study. ***Arch Pharm Res*** 2012; 35: 1817-1822.