POLYMORPHISM OF ANGIOTENSIN II TYPE 1RECEPTOR GENE IN ESSENTIAL HYPERTENSION IN IRANIAN POPULATION

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ABSTRACT

Renin angiotensin system (RAS) has an important role in the regulation of hypertension. RAS includes angiotensinogen, Angiotensin Converting Enzyme (ACE), angiotensine II and angiotensin receptors (AGTR). Angiotensin receptors have several types but AT1R is the main subtype. In this study the effect of A1166→C polymorphism of AT1R gene and the role of possible genetic differences in hypertension was investigated. DNA of the whole blood leukocytes from hypertensive patients and healthy people of Mashhad population as control, were extracted and then PCR was performed on prepared samples followed by amplification of the target fragments which were then digested with the *Dde*I restriction enzyme. Data were classified on the basis of genotypes and gender and then alleles and genotypes frequencies were analyzed statistically. There were no significant differences in the genotype, and allele frequencies between hypertensive and normotensive subjects. However, frequency of C allele of AT1R gene in hypertensive women was significantly higher than normotensive women ($P<0.05$). These results suggest that C allele of AT1R gene may be an important risk factor for essential hypertension in women.

Keywords: Angiotensin, Gene polymorphisms, Hypertension

INTRODUCTION

Cardiovascular diseases are increasing in epidemic proportions in developing countries and are the leading cause of death in industrialized countries (1, 2). The main risk factors, such as arterial hypertension, diabetes mellitus and hyperlipidemia are affected by genetic and environmental factors. Rennin-angiotensin system genes are among the most important factors for investigation of the cause of hypertension. Angiotensin II acts mainly via the angiotensin II type 1 receptor (AT1R) as a potent vasoconstrictor which regulates vascular tone and systemic blood pressure. A polymorphism in the 3'-untranslated region of AT1R gene (A1166C) has been linked with essential hypertension (3-7). It also affects responses to losartan (8) antidepressants (9) and angiotensin II (10). While this gene polymorphism is associated with cardiac hypertrophy (11) and increased artery vasoconstriction (12, 13), it is difficult to interpret this association since the polymorphic variation is found in the non coding region of the gene. Recently it has been suggested that the A1166C polymorphism may be involved in the regulation of the expression of AT1R gene (8). Interestingly a weak but significant linkage disequilibrium with a polymorphism in the promoter region of the AT1R gene and AT1R/A1166C has also been reported (14). Interethnic differences in cardiovascular diseases indicate the need to examine the association of AT1R gene polymorphism and hypertension in other populations. The present study was conducted to determine relationship between essential hypertension and AT1R polymorphism in Iranian population.

MATERIALS AND METHODS

The DNA amplification reaction was performed using a Touchgene Gradient Thermal Cycler (Techne, Cambridge, England). All enzymes and chemicals used in this study were of molecular grade, either from Sigma, MBI Fermentas, or Promega.

Patients

The hypertensive group was 74 subjects consisting of 41 men and 33 women. Using clinical and laboratory examinations, secondary forms of hypertension were excluded from this study. The control group was 91 normotensive subjects consisting of 54 men and 37 women. The control group had no family history of cardiovascular diseases. Demographic, clinical characteristics and blood chemistry data were obtained from questionnaires, and by clinical and laboratory examinations.

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Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using a standard protocol (15). All The polymorphism studies of AT1R gene were conducted by polymerase chain reaction (PCR). The primer design and reported polymorphisms were in the order of the genomic sequence of GenBank entry AF245699 (NCBI). The A1166C variant of the AT1R gene was identified with primers: 5'-GCACCATGTTTTGAGGTTG -3' as the forward and 5'-CGACTACTGCTTAGCATA-3' as the reverse primers under the conditions described elsewhere (16, 17). Briefly, for a 50 µL PCR, the reaction contained 200 ng genomic DNA, 200 µmol/l of each of dNTPs (dATP, dCTP, dGTP and dTTP), 250 ng of each primer, 1.5 mmol/l magnesium chloride and 1 U *Taq* DNA polymerase (MBI Fermentas). PCR amplification consisted of an initial 5 min denaturation at 94 ^{o}C, followed by 35 cycles of denaturation at 94 ^{o}C for 30 s, annealing at 55 ^{o}C for 30 s, and extension at 72 ^{o}C for 60 s. The terminal extension was performed at 72 ^{o}C for 10 min. The PCR product was digested with the *Dde*I restriction enzyme (Promega). Digested products were separated by agarose gel electrophoresis and visualized directly under UV light after staining with ethidium bromide. Undigested 540 bp fragment indicated the presence of the A allele and, appearance of two bands at 110 and 430 bp represented the C allele.

Statistical analysis

Statistical analysis using Instat version 3 included the χ^2 test for genotype and allele frequencies comparison. Mean values were compared between hypertensive and control subjects by the unpaired student's t- test. A level of $P < 0.05$ was considered statistically significant.

RESULTS

In vitro DNA amplification of the AT1R gene using the specific primers resulted in a 540 bp DNA product (Figure 1A). On digestion of the amplified fragment (amplicon) with *Dde*I restriction endonuclease, DNA fragments of 540 (AA), 430 (CC) or 540 and 430 (AC) bp length were observed. Thus each of samples revealed one of the three different electrophoretic patterns (Figure 1B). Frequencies of the AA, AC and CC genotypes were 42.4, 10.9 and 1.8 in normotensives and 30.3, 12.1 and 2.5 in hypertensives, respectively. Frequencies for genotypes and alleles in the study population are presented in Table 1. The clinical and biochemical parameters of the control and hypertensive subjects are shown in Table 2. When the study subjects were divided into male and female subgroups, the distribution of the A1166C genotype were different significantly in females ($P = 0.033$), but there were no differences in males (Table 3). Frequency of AT1R C allele carriers was found higher in female hypertensives than controls (8.4 % vs 3.7 %, $P = 0.033$). In male subjects this ratio was 6.3% in hypertensives and 8.9% in controls (Table 3, $P = 0.99$).

DISCUSSION

Hypertension is one of the major risk factors for cardiovascular diseases. The prevalence of hypertension is increasing worldwide (1).

According to a recent study, in some cities of Iran, the incidence of hypertension is relatively high, ranging from 7.7 % in Boshehr up to 28.8 % in Hamadan (18). In the city of Isfahan, hypertension was found to affect 16.8% males and 19.4% female subjects (18). Another study conducted in Tehran between 1999 and 2000 indicated that among the population studied, 23% of women and 20% of men were hypertensive (19).

Human essential hypertension has been linked to genetic and environmental factors with genetic susceptibility being responsible for 30 to 50 % of the phenotype expression (20). Many polymorphisms of the AT1R gene have been reported, where an A to C substitution at position 1166 being the most widely studied (3, 21-23).

Genetic studies have indicated that in many ethnic populations the substitution of cytosine for adenine at position 1166 was associated with susceptibility to essential hypertension (3, 4, 23-25). No data has been reported so far on the possible effect of A1166C polymorphism of the AT1R gene on the blood pressure of Iranians.

In this study, the association between AT1R gene polymorphism (A1166C) and hypertension in an Iranian population in Mashhad was investigated. It was found that female subjects with C1166 allele had a higher genetic predisposition to hypertension compared to AA homozygotes. However, this association was not observed in the male population. These results seem to be consistent with the previous reports that indicated an association between C1166 and essential hypertension. The C allele of the A1166C polymorphism has been shown by several studies to be associated with the severe form of essential hypertension, but the role of this polymorphism is

still ambiguous in pathologies related to high Angiotensin II levels, such as deterioration of

Table1. Frequencies of genotypes and alleles in the whole population

		Hypertensive (%)	Controls (%)	
Genotype frequencies				
	AA	50 (30.3)	70 (42.4)	
	AC	20 (12.1)	18 (10.9)	
	CC	4 (2.5)	3 (1.8)	
Significance		$\chi^2_{(df=1)}$= 1.85	P = 0.39 > 0.05	NS
Allelic frequencies				
	A allele	120 (36.4)	158 (47.9)	
	C allele	28 (8.5)	24 (7.3)	
Significance		$\chi^2_{(df=1)}$= 2.02	P= 0.155 > 0.05	NS

Values are counts, with the relative percentage of each group in parentheses. Instat version 3 was used for statistical analyses between hypertensive and control subjects. A level of $P < 0.05$ was considered statistically significant.

Table 2. Mean clinical and biochemical characteristics of AT1R genotypes in total subjects

	Hypertensive cases						Controls					
	Women			Men			Women			Men		
	AA	AC	CC	AA	AC	CC	AA	AC	CC	AA	AC	CC
Age (yr)	58.5	52.7	54	54.6	49.0	50.0	44.9	42.5	-*	44.3	44.6	38
Choleterol (mg/dL)	231	218	244	229	240	226	206	237	-	202	182	216
LDL-Cholestrol (mg/dL)	128	132	205	148	137	111	133	142		129	103	176
HDL-Cholestrol (mg/dL)	44	45	49	42	42	46	47	38	-	49	53	34
Triglyceride (mg/dL)	178	197	157	136	117	342	164	117	-	177	157	98

*not available

Values are mean measures for each biological parameter. LDL and HDL indicate low-density lipoprotein and high-density protein respectively.

Table 3. Distribution of A1166C variants of AT1R in the study population

	Hypertensive (%)			Controls (%)			Significance	
Genotypic frequencies:	AA	AC	CC	AA	AC	CC	X^2	P
Men (total 95)	30 (31.5)	10 (10.5)	1 (1.1)	39 (41.1)	13 (13.7)	2 (2.1)	0.12	0.94 (NS)*
Women (total 70)	20 (28.8)	10 (14.3)	3 (4.3)	31 (44.3)	5 (7.1)	1 (1.4)	4.83	0.09 (NS)
Allelic variation	A Allele		C Allele	A Allele		C Allele	X^2	P
Men	70 (36.8)		12 (6.3)	91 (47.9)		17 (8.9)	4.3x 10^{-5}	0.99 (NS)
Women	50 (26.3)		16 (8.4)	67 (35.3)		7 (3.7)	4.53	0.033 (S)**

* Not significant

** Significant

Values are counts, with the relative percentage of each group in parentheses. Instat version 3 was used for statistical analyses between hypertensive and control subjects divided by gender. A level of $P < 0.05$ was considered statistically significant.

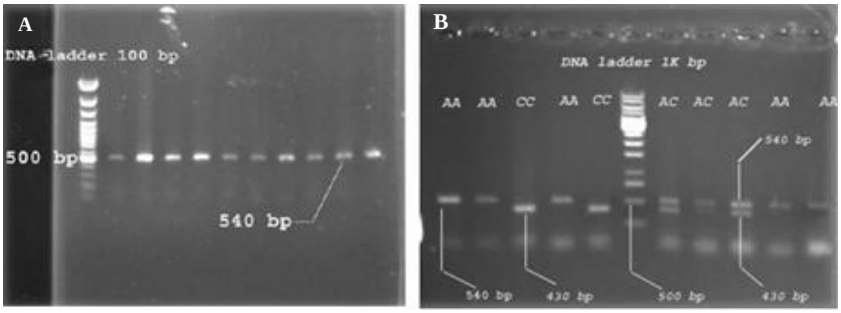

Figure 1. A. Amplification of the 540 bp fragment of human AT1R gene**.** The first lane from left is DNA ladder marker and all the other lanes represent the 540 bp PCR product (amplicon). **B.** A1166C polymorphism of the AT1R gene. DNA ladder marker in central lane is labeled. The photo shows all three genotypes obtained from study cases (AA, AC and CC) seen on the ethidium bromide-stained gel. These patterns were observed in both control and patient groups with the frequencies reported in Tables 1 and 3. The homozygous (AA) has a band of 540 bp, the homozygous (CC) has a band of 430 bp and the heterozygous (AC) has two band

renal function, arterial stiffness and hypertrophic cardiomyopathy (5,8,26). The molecular mechanism of involvement of the C to A substitution in AT1R gene in hypertension is still not clear, since the variable nucleotides are located in the 3' untranslated region of the gene (21). It is suggested that this polymorphic variation could be involved in the regulation of AT1R gene expression (8). It has been demonstrated that a weak but significant linkage disequilibrium between A1166C poly-morphism with a polymorphism (810AV) in the promoter region of the AT1R gene suggesting that the A1166C polymorphism could be associated with regulation of expression of AT1R gene (14).

Substantial evidence has been gathered that indicate there is gender difference in RAS activity which may account for differences in blood pressure of men and women (16, 17, 27, 28). Some components of the RAS are regulated by estrogen as the angiotensinogen gene promoter and demonstrate estrogen-responsiveness (29).

It has been suggested that there is interaction between gender and A1166→C gene polymorphism which affects blood pressure. The allele C1166 has been reported to be linked with preeclampsia (30). It was shown that the AC/CC genotype was associated with higher blood pressure values than the AA genotype in women (17).

CONCLUSION

In summary, an increased risk for hypertension in women carrying the C1166 allele was observed. The number of participants (patients and controls) included in this study is probably not enough to draw definite conclusions. However these results are in agreement with previously reported association between A1166C polymorphism of AT1R gene and risk of the development of hypertension in females. Further studies on larger population samples and other ethnic groups will be useful for elucidation of the linkage between AT1R polymorphism and risk of hypertension.

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REFERENCES

1. Beaglehole, R. International trends in coronary heart disease mortality and incidence rates. J. Cardiovasc. Risk. 1999; 6(2): 63-8.
2. Lusis, A.J. Atherosclerosis. Nature 2000; 407(6801): 233-41.
3. Bonnardeaux, A., Davies, E., Jeunemaitre, X., Fery, I., Charru, A., Clauser, E., et al. Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. Hypertension 1994; 24: 63-9.
4. Wang, W.Y., Zee, R.Y., Morris, B.J. Association of angiotensin II type 1 receptor gene polymorphism with essential hypertension. Clin. Genet. 1997; 51(1): 31-4.
5. Zhang, K.X., Liu, T.B., Xu, Q.X., Zhu, D.L., Huang, W. Association of angiotensin II receptor type 1 gene single nucleotide polymorphism with Chinese essential hypertension complicated with coronary heart disease. Zhonghua Xin Xue Guan Bing Za Zhi 2005; 33(8): 720-3.
6. Henskens, L.H., Kroon, A.A., van Boxtel, M.P., Hofman, P.A., de Leeuw, P.W. Associations of the angiotensin II type 1 receptor A1166C and the endothelial NO synthase G894T gene polymorphisms with silent subcortical white matter lesions in essential hypertension. Stroke 2005; 36(9): 1869-73.
7. Sugimoto, K., Katsuya, T., Ohkubo, T., Hozawa, A., Yamamoto, K., Matsuo, A., et al. Association between angiotensin II type 1 receptor gene polymorphism and essential hypertension: the Ohasama Study. Hypertens. Res. 2004; 27(8): 551-6.

8. Sookoian, S., Castano, G., Garcia, S.I., Viudez, P., Gonzalez, C., Pirola, C.J. A1166C angiotensin II type 1 receptor gene polymorphism may predict hemodynamic response to losartan in patients with cirrhosis and portal hypertension. Am. J. Gastroenterol. 2005; 100(3): 636-42.
9. Bondy, B., Baghai, T.C., Zill, P., Schule, C., Eser, D., Deiml, T., et al. Genetic variants in the angiotensin I-converting-enzyme (ACE) and angiotensin II receptor (AT1) gene and clinical outcome in depression. Prog Neuropsychopharmacol Biol. Psychiatry 2005; 29(6): 1094-9.
10. van Geel, P.P., Pinto, Y.M., Voors, A.A., Buikema, H., Oosterga, M., Crijns, H.J., et al. Angiotensin II type 1 receptor A1166C gene polymorphism is associated with an increased response to angiotensin II in human arteries. Hypertension 2000; 35(3): 717-21.
11. Osterop, A.P., Kofflard, M.J., Sandkuijl, L.A., ten Cate, F.J., Krams, R., Schalekamp, M.A., et al. AT1 receptor A/C1166 polymorphism contributes to cardiac hypertrophy in subjects with hypertrophic cardiomyopathy. Hypertension 1998; 32(5): 825-30.
12. Amant, C., Hamon, M., Bauters, C., Richard, F., Helbecque, N., McFadden, E.P., et al. The angiotensin II type 1 receptor gene polymorphism is associated with coronary artery vasoconstriction. J. Am. Coll. Cardiol. 1997; 29(3): 486-90.
13. Henrion, D., Amant, C., Benessiano, J., Philip, I., Plantefeve, G., Chatel, D., et al. Angiotensin II type 1 receptor gene polymorphism is associated with an increased vascular reactivity in the human mammary artery in vitro. J. Vasc. Res. 1998; 35(5): 356-62.
14. Erdmann, J., Riedel, K., Rohde, K., Folgmann, I., Wienker, T., Fleck, E., et al. Characterization of polymorphisms in the promoter of the human angiotensin II subtype 1 (AT1) receptor gene. Ann. Hum. Genet. 1999; 63 (Pt 4): 369-74.
15. Lahiri, D.K., Nurnberger, J.I., Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res. 1991; 19(19): 5444.
16. Miller, J.A., Anacta, L.A., Cattran, D.C. Impact of gender on the renal response to angiotensin II. Kidney Int. 1999; 55(1): 278-85.
17. Reich, H., Duncan, J.A., Weinstein, J., Cattran, D.C., Scholey, J.W., Miller, J.A. Interactions between gender and the angiotensin type 1 receptor gene polymorphism. Kidney Int. 2003; 63(4): 1443-9.
18. Sarraf-Zadegan, N., Boshtam, M., Mostafavi, S., Rafiei, M. Prevalence of hypertension and associated risk factors in Isfahan, Islamic Republic of Iran. East Mediterr. Health J. 1999; 5(5): 992-1001.
19. Azizi, F., Ghanbarian, A., Madjid, M., Rahmani, M. Distribution of blood pressure and prevalence of hypertension in Tehran adult population: Tehran Lipid and Glucose Study (TLGS), 1999-2000. J. Hum. Hypertens. 2002; 16(5): 305-312.
20. Munroe, P.B., Caulfield, M.J. Genetics of hypertension. Curr. Opin. Genet. Dev. 2000; 10(3): 325-9.
21. Duncan, J.A., Scholey, J.W., Miller, J.A. Angiotensin II type 1 receptor gene polymorphisms in humans: physiology and pathophysiology of the genotypes. Curr. Opin. Nephrol. Hypertens. 2001; 10(1): 111-6.
22. Paillard, F., Chansel, D., Brand, E., Benetos, A., Thomas, F., Czekalski, S., et al. Genotype-phenotype relationships for the renin-angiotensin-aldosterone system in a normal population. Hypertension 1999; 34(3): 423-9.
23. Kainulainen, K., Perola, M., Terwilliger, J., Kaprio, J., Koskenvuo, M., Syvanen, A.C., et al. Evidence for involvement of the type 1 angiotensin II receptor locus in essential hypertension. Hypertension 1999; 33(3): 844-9.
24. Perola, M., Kainulainen, K., Pajukanta, P., Terwilliger, J.D., Hiekkalinna, T., Ellonen, P., et al. Genome-wide scan of predisposing loci for increased diastolic blood pressure in Finnish siblings. J. Hypertens. 2000; 18(11): 1579-85.
25. Agachan, B., Isbir, T., Yimaz, 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(6): 545-549.
26. Baudin, B. Polymorphism in angiotensin II receptor genes and hypertension. Exp. Physiol. 2005; 90(3): 277-82.
27. Ellison, K.E., Ingelfinger, J.R., Pivor, M., Dzau, V.J. Androgen regulation of rat renal angiotensinogen messenger RNA expression. J. Clin. Invest. 1989; 83(6): 1941-5.
28. James, G.D., Sealey, J.E., Muller, F., Alderman, M., Madhavan, S., Laragh, J.H. Renin relationship to sex, race and age in a normotensive population. J. Hypertens. Suppl. 1986; 4(5): S387-9.
29. Gordon, M.S., Chin, W.W., Shupnik, M.A. Regulation of angiotensinogen gene expression by estrogen. J. Hypertens. 1992; 10(4): 361-6.
30. Bouba, I., Makrydimas, G., Kalaitzidis, R., Lolis, D.E., Siamopoulos, K.C., Georgiou, I. Interaction between the polymorphisms of the renin-angiotensin system in preeclampsia. Eur. J. Obstet. Gyn. R B 2003; 110(1): 8-11.