Correlation between angiotensinogen gene and primary hypertension with cerebral infarction in the Li nationality of China

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Abstract: Objective To investigate the relationship of four single nucleotide polymorphism (SNP) haplotypes in the angiotensinogen (*AGT*) gene to the primary hypertension with or without cerebral infarction in the Li nationality of Hainan, China. **Methods** Total 300 subjects were allocated into three different groups: Group 1, 100 patients who have primary hypertension; Group 2, 100 patients who have primary hypertension with cerebral infarction; and control group, 100 healthy individuals. The genotypes of all subjects were determined by PCR-sequencing to analyze the four polymorphisms at position -152 (G-A), -20 (A-C), -18 (C-T), and -6 (A-G) in the promoter region of *AGT*. **Results** The frequencies of CT genotype of *AGT*-18 and T allele in Group 1 ($P = 0.003$, $P = 0.004$) and Group 2 ($P = 0.002$, $P = 0.002$) were both significantly higher than in healthy controls. The frequency of G allele of *AGT*-6 was significantly higher in Group 2 than in the control group ($P = 0.016$), while there is no significant difference between Group 1 and the control. Haplotype analysis revealed that H6 haplotype frequency which included -20C and -6G was significantly increased in Group 2 ($P = 0.003$) compared with the control group, while H5 haplotype frequency which included -20C and -18T was significantly increased in Group 1 ($P = 0.006$) versus the control. **Conclusion** The -20 (A-C) and -18 (C-T) of the *AGT* may play an important role in pathogenesis of primary hypertension; and -20 (A-C), -18 (C-T), and -6 (A-G) may be the genetic risk factors for the onset of primary hypertension with cerebral infarction in the Li nationality of Hainan, China.

Keywords: angiotensinogen gene; primary hypertension; cerebral infarction; single nucleotide polymorphism; haplotype

1 Introduction

Cerebrovascular diseases are the second leading cause of death for human beings at present, and approximately 60%-70% of them are ischemic stroke. Brain ischemia may induce neuron necrosis or apoptosis. Though the mechanism is not very clear, researches indicate that apoptosis may be related to the augment of oxygen free radical, calcium overload, and the release of the excitatory amino acids following the reperfusion^[1]. Ischemic stroke is highly related with hypertension, and other risk factors such as smoking, drinking, diabetes and hypercholesteremia also play important roles.

Renin-angiotensin system (RAS) is not only important for water-electrolyte balance, but also play key roles in maintaining blood pressure and promoting the growth and proliferation of cardiovascular cells. Molecular genetics studies reveal that RAS also takes part in the pathogenesis of cardio-cerebrovascular diseases. Consequently angiotensinogen gene (*AGT*), the only incipient substrate of angiotensin precursor, is widely observed as one of the genes associated to cerebrovascular diseases.

A lot of researches have indicated that mutations at a single site on the *AGT* gene are associated to essential (primary) hypertension^[2,3]. The recently researches on the relation between *AGT* polymorphism and primary hypertension in various nationalities of China have demonstrated that the single nucleotide polymorphism (SNP) sites in the *AGT* gene are different among races and individuals, and result in their different susceptibility to diseases^[4].

The purpose of present study was to survey the rela-

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tionship between the polymorphisms of -152 (G-A), -20 (A-C), -18 (C-T), and -6 (A-G) sites in the promoter region of *AGT* and the primary hypertension combined with cerebral infarction, so as to define the roles of *AGT* in the pathogenesis of cerebrovascular diseases in the Li nationality of Hainan Province, China.

2 Materials and methods

2.1 Subjects All subjects were Li nationalities living for several generations in the Ledong County and Changjiang County in Hainan Province. They were randomly selected from the healthy population (control group) and the in-patients/outpatients (the primary hypertension combined with cerebral infarction group, PHACI) in the county hospital of these two counties from July 2005 to September 2006.

They were allocated into three groups. Group 1 was the essential hypertension group with 100 patients (51 males and 49 females), who were all diagnosed as essential hypertension according to the diagnostic criteria for hypertension (WHO, 1999), i.e. the systolic pressure ≥ 140 mmHg and/or the diastolic pressure ≥ 90 mmHg. Blood pressure was measured at rest state for three times. In addition, we had excluded the subjects with secondary hypertension, diabetes, coronary heart disease, and severe liver, kidney or thyroid diseases. Group 2 was the essential hypertension with cerebral infarction group, consisting of 100 patients (52 males and 48 females) with definite cerebral infarction determined by clinical examination and skull CT (and/or MRI) (based on the Diagnostic Criteria for Cerebrovascular Diseases at the fourth National Conference on Cerebrovascular Diseases of China). Group 1 and Group 2 constituted the PHACI group. The third group was the normal control consisted of 100 (50 males and 50 females) healthy people. The average ages for Group 1, Group 2, and control were 54.16 ± 11.09 years, 51.37 ± 11.25 years, and 52.68 ± 10.87 years, respectively.

2.2 Genome DNA Extraction Five-milliliter of peripheral blood was anticoagulated with 3.8% sodium citrate (1:6). Genome DNA was extracted from leukocyte by phenol/chloroform method and was dissolved in TE.

2.3 Detection of SNPs in the promoter region of *AGT* gene PCR primers were synthesized by Beijing Tsingke Biotechnology Co. (China). The primer sequences are 5'-CCCTGCACCGGCTCACTCTG-3' for PCR, and 5'-GCA GGAAGACCA GAC CAT CT-3' for reverse PCR.

2.4 Statistical analysis In both Group 1 and Group 2, the fitness with Hardy-Weinberg equilibrium law of SNP gene, parameters of site linkage disequilibrium (LD) (D'), and LD of polymorphic sites distribution were analyzed by SHEsis software^[5]. The differences in frequency distributions of allele and genotype among groups were analyzed by χ^2 test. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Clinical data comparison in PHACI group and control group There was no significant difference in sex and age among three groups ($P = 0.405$, $P = 0.980$).

3.2 SNP distribution The frequency distributions of the polymorphic genotypes (AA, AG, or GG) and A/G allele at the Site -152 (G-A) had no significantly difference between Group 1 and the healthy control ($\chi^2 = 0.166$, $P = 0.684$ and $\chi^2 = 0.154$, $P = 0.695$), the same as Group 2 with the healthy control ($\chi^2 = 2.407$, $P = 0.12$ and $\chi^2 = 2.260$, $P = 0.133$) and Group 2 with Group 1 ($\chi^2 = 1.330$, $P = 0.249$ and $\chi^2 = 1.256$, $P = 0.262$).

The frequency distributions of the polymorphic genotypes (CC, AC, or AA) and C/A allele at the Site -20 (A-C) between Group 1 and the healthy controls were not significantly different ($\chi^2 = 1.729$, $P = 0.421$ and $\chi^2 = 0.345$, $P = 0.557$), the same as the Group 2 with the healthy controls ($\chi^2 = 0.328$, $P = 0.848$ and $\chi^2 = 0.237$, $P = 0.626$) and Group 2 with Group 1 ($\chi^2 = 2.927$, $P = 0.231$ and $\chi^2 = 1.152$, $P = 0.283$).

For the polymorphic genotypes (TT, CT, or CC) and the T allele at the Site -18 (C-T), the frequency distributions in Group 1 ($\chi^2 = 8.721$, $P = 0.003$ and $\chi^2 = 8.381$, $P = 0.004$) and Group 2 ($\chi^2 = 9.783$, $P = 0.002$ and $\chi^2 = 9.375$, $P = 0.002$) were significantly higher than the healthy control, but we had not yielded the same result between the Group 2 and the Group 1 ($\chi^2 = 0.043$, $P = 0.836$ and $\chi^2 = 0.039$, $P = 0.842$).

The same as the first two genotypes, the frequency distributions of the polymorphic genotypes (GG, GA, or AA) and the G/A allele at the Site -6 (A-G) had no significant difference between Group 1 and the healthy control ($\chi^2 = 1.542$, $P = 0.462$ and $\chi^2 = 1.731$, $P = 0.191$) and between Group 2 and Group 1 ($\chi^2 = 1.139$, $P = 0.566$ and $\chi^2 = 1.226$, $P = 0.268$). In Group 2, the frequency distribution of G allele at Site (-6) was significantly higher than that in the healthy control, though the polymorphic genotypes had no difference. (Tab. 1)

3.3 Haplotype distribution in SNP composition The distributions of the haplotype of *AGT* gene in Group 1 and Group

Tab. 1 SNP distribution

Group	Genotype (%)			χ^2	<i>P</i>	Allele frequency (<i>n</i>)		χ^2	<i>P</i>
-152G/A	A/A	A/G	G/G			A	G		
Control	0.0	15.0	85.0	0.166*	0.684*	0.075 (15)	0.925 (185)	0.154*	0.695*
Group 1	0.0	13.0	87.0	2.407 [#]	0.121 [#]	0.065 (13)	0.935 (187)	2.260 [#]	0.133 [#]
Group 2	0.0	8.0	92.0	1.330 ^{&}	0.249 ^{&}	0.040 (8)	0.960 (192)	1.256 ^{&}	0.262 ^{&}
-20A/C	C/C	A/C	A/A			C	A		
Control	3.0	39.0	58.0	1.729*	0.421*	0.225 (45)	0.775 (155)	0.345*	0.557*
Group 1	7.0	36.0	57.0	0.328 [#]	0.848 [#]	0.250 (50)	0.750 (150)	0.237 [#]	0.626 [#]
Group 2	2.0	7.0	61.0	2.927 ^{&}	0.231 ^{&}	0.205 (41)	0.795 (159)	1.152 ^{&}	0.283 ^{&}
-18C/T	T/T	C/T	C/C			T	C		
Control	0.0	2.0	98.0	8.721*	0.003*	0.010 (2)	0.990 (198)	8.381*	0.004*
Group 1	0.0	13.0	87.0	9.783 [#]	0.002 [#]	0.065 (13)	0.935 (187)	9.375 [#]	0.002 [#]
Group 2	0.0	14.0	86.0	0.043 ^{&}	0.836 ^{&}	0.070 (14)	0.930 (186)	0.039 ^{&}	0.842 ^{&}
-6A/G	G/G	A/G	A/A			G	A		
Control	1.0	10.0	89.0	1.542*	0.462*	0.060 (12)	0.940 (188)	1.731*	0.191*
Group 1	2.0	15.0	83.0	5.201 [#]	0.074 [#]	0.095 (19)	0.905 (181)	5.699 [#]	0.016 [#]
Group 2	3.0	20.0	77.0	1.139 ^{&}	0.566 ^{&}	0.130 (26)	0.870 (174)	1.226 ^{&}	0.268 ^{&}

Group 1: the essential hypertension group; Group 2: the essential hypertension group with cerebral infarction. *Group 1 vs Control; [#]Group 2 vs Control; [&]Group 1 vs Group 2.

Tab. 2 Haploid distribution in SNP composition (Group 1 vs Control)

Haplotype*	Group 1		Control		χ^2	<i>P</i>	Odds Ratio [95% CI]
	number	frequency	number	frequency			
H1 (0000)	125	0.627	143	0.715	3.521	0.061	0.669 [0.440–1.019]
H2 (0100)	30	0.150	28	0.140	0.086	0.769	1.087 [0.623–1.897]
H3 (1100)	13	0.063	15	0.075	0.229	0.633	0.828 [0.381–1.799]
H4 (0001)	19	0.095	10	0.050	3.060	0.080	2.008 [0.908–4.442]
H5 (0110)	7	0.035	0	0.000	7.125	0.008	—

*The allele order is -152 (G-A), -20 (A-C), -18 (C-T), and -6 (A-G) from left to right. Left allele is indicated by 0 and right allele is by 1.

Tab. 3 Haploid distribution in SNP composition (Group 2 vs Control)

Haplotype*	Group 2		Control		χ^2	<i>P</i>	Odds Ratio [95% CI]
	number	frequency	number	frequency			
H1 (0000)	134	0.672	143	0.715	0.896	0.344	0.814 [0.532–1.247]
H2 (0100)	18	0.088	28	0.140	2.610	0.106	0.597 [0.317–1.122]
H3 (1100)	8	0.040	15	0.075	2.260	0.133	0.514 [0.213–1.241]
H4 (0001)	13	0.063	10	0.050	0.350	0.554	1.294 [0.550–3.041]
H5 (0010)	12	0.060	2	0.010	7.402	0.006	6.319 [1.396–28.612]
H6 (0101)	13	0.067	2	0.010	8.578	0.003	6.849 [1.560–30.073]

*The allele order is -152 (G-A), -20 (A-C), -18 (C-T), and -6 (A-G) from left to right. Left allele is indicated by 0 and right allele is by 1.

2 were 5 haplotypes and 6 haplotypes, respectively. Among them, four haplotypes which were shared in common by both Group 1 and Group 2 can explain about 90% of the chromosomes. The frequencies of these 4 haplotypes had no significant differences between PHACI group and the control ($P > 0.05$). The frequency distribution of the H5 haplotype which consisted of -152G, -20C, -18T, and -6A ($7/0$, $\chi^2 = 7.125$, $P = 0.008$) was significantly higher in the Group 1 than in the control. Meanwhile, the frequency distributions of the H6 haplotype which consisted of -152G, -20C, -18C, and -6G and the H5 haplotype which consisted of -152G, -20A, -18T, and -6A ($13/2$, $\chi^2 = 8.578$, $P = 0.003$; $12/2$, $\chi^2 = 7.402$, $P = 0.006$) were significantly higher in Group 2 than in the corresponding controls. (Tab. 2, 3)

4 Discussion

Cerebral infarction is a complex disease which can be influenced by hereditary factors and environmental conditions (possibly with greater significance for the former). Thus, many researchers surveyed the correlation between cerebral infarction and gene polymorphism. The result of the largest-so-far summary analysis on 51 researches and 1 million population suggests that systolic pressure and diastolic pressure are independent predictors for cerebral apoplexy, and the significance of pulse pressure for cerebral apoplexy increases to some extent for persons beyond 55 years old^[6]. Therefore, it is especially important to study the pathogenesis factors of PHACI.

RAS system is universally recognized for its important roles in the pathogenesis of primary hypertension. AGT is the only known incipient substrate of RAS system. Upstream core promoter domain (AG core promoter element 1, AGCE1) was necessary for *AGT* transcription. AGCE1 might control blood pressure by regulating the expression of *AGT* gene and affecting AGT level in blood circulation^[7]. As reported by Inoue *et al.*^[8], G-6A mutation of AGCE1 could increase the basal transcription rate of *AGT* gene by about 30%-70%.

There are only a few known hereditary characteristics of cerebral infarction. Some reports^[9,10] showed that 235TT polymorphism of *AGT* gene was significantly positively related to the pathogenesis of cerebral apoplexy in Chinese population; and M235T polymorphism of *AGT* gene might be the hereditary risk factor for PHACI in Chinese Han nationality. The present study first analyzed the correlation between the PHACI and the polymorphism and

haplotypes of four sites [-152 (G-A), -20 (A-C), -18 (C-T), and -6 (A-G)] in the promoter region of *AGT* gene in the Li nationality. In all of the three groups, the frequency distributions of genotypes at these four sites were of good fitness with H-W equilibrium ($P > 0.05$), indicating that these genotypes from the same population had reached hereditary equilibrium of group representativeness.

The outcomes about *AGT*-18 site in different studies are diverse. There was no -18 (C-T) mutation among the mutations of *AGT* gene in Kazak population^[11]. In the research of Sato *et al.* in Japanese population^[12], however, the frequency of CC genotype with -18 (C-T) mutation increased significantly in hypertension, indicating that this mutation was related to hypertension. In the present study, the frequency distributions of the TT, CT, and CC genotypes and T/C gene of the *AGT*-18 site were significantly different among the three groups, indicating that the C-T mutation at Site (-18) might be related to the PHACI in the Li nationality. The frequency of CT genotype with -18 (C-T) mutation increased significantly in primary hypertension. The inconsistent results among these researches showed different gene mutations in different population. The frequency of G/A allele at Site (-6) was significantly different among the three groups, indicating that G allele at Site (-6) might increase the risk for PHACI. Morgan and Humphrise reviewed on apoplexy gene after October 2003^[13] that most researches supported that the polymorphism of *AGT* gene was not related to ischemic cerebrovascular diseases. Inconsistent with this conclusion, however, the result of the present study showed that the polymorphism in the promoter region of *AGT* gene was one of possible reasons for PHACI in the Li nationality.

The analysis is more powerful to focus on the transmission of multilocus haplotypes, as opposed to alleles at individual loci^[14], thus the combined analysis of multiple SNPs is of greater hereditary significance than analysis of single site. As shown in our haploid analysis, four polymorphic sites constituted six haplotypes; and the frequency distributions for the H6 haplotype consisting of G-C-C-G allele and the H5 haplotype consisting of G-A-T-A allele were significantly higher in the PHACI group than in the control group, indicating that H6 and H5 haplotypes may be related to PHACI pathogenesis. Gene mutation was occurred at Site -18T but not at other sites for H5 haplotype; and the -18(C-T) mutation may be related to PHACI pathogenesis in the Li nationality as shown by the SNP analysis

at single site. Thus, PHACI might be caused primarily by the -18 (C-T) mutation of H5 haplotype but seldom by that of allele at Site -152, -20, and -6. Moreover, H6 haplotype consisted of the alleles at Site -20 and -6. Therefore, PHACI may be caused by the synergic action of the polymorphic sites, such as influencing the transcription activity of *AGT* gene, changing the concentration of plasma AGT, changing the activity of local rennin-angiotensin system, and changing the expression of AGT in local brain tissues. H6 haplotype may be an important hereditary factor for PHACI pathogenesis in the Li nationality.

To sum up, in core promoter region at the 5' end of *AGT* gene, the mutation at Site C-18T may be related to the susceptibility of PHACI in the Li nationality, and may be one of its possible reasons; G allele at Site -6 may increase the risk for PHACI onset; and H6 haplotype (at Site -20C and -6G) may be an important hereditary factor for PHACI pathogenesis in the Li nationality.

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血管紧张素原基因与黎族原发性高血压并脑梗死的相关性

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摘要: **目的** 研究血管紧张素原基因(angiotensinogen, *AGT*)4个位点的单核苷酸多态及其构成的单倍型与海南黎族原发性高血压及原发性高血压并发脑梗死的相关性。**方法** 选择海南黎族人群为研究对象, 利用PCR直接测序法, 在100例原发性高血压患者、100例原发性高血压并脑梗死患者和100例健康对照者中, 对*AGT*基因启动子区域的-152(G-A), -20(A-C), -18(C-T), -6(A-G)多态进行基因分型。**结果** C-18T多态的基因型和T等位基因频率分布在原发性高血压组($P=0.003$, $P=0.004$)和原发性高血压并脑梗死组($P=0.002$, $P=0.002$)均比健康对照组显著增高。-6(A-G)多态的G等位基因频率在原发性高血压并脑梗死组显著高于健康对照组($P=0.016$), 而与原发性高血压组比较无显著性差异。单倍型分析提示, 由-20C和-18T构成的H5单倍型在原发性高血压组中增加, 与对照组相比有显著性差异($P=0.006$); 而由-20C和-6G构成的H6单倍型在原发性高血压并脑梗死组中明显增加, 与对照组相比有显著性差异($P=0.003$)。**结论** 在海南黎族人群中, *AGT*基因的-20(A-C), -18(C-T)多态可能对原发性高血压的发病起了重要作用; -20(A-C), -18(C-T)和-6(A-G)多态可能是原发性高血压并脑梗死发病的遗传危险因素。

关键词: 血管紧张素原基因; 原发性高血压; 脑梗死; 单核苷酸多态; 单倍型