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Racial difference in aldose reductase C-106T genetic polymorphism and association with essential hypertension

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ABSTRACT

Objective: To investigate the distribution of aldose reductase (AR) C-106T genetic polymorphism in Chinese Han population and its association with the risk for essential hypertension (EH).

Methods: The AR C-106T polymorphism was genotyped in 148 Chinese EH patients and 137 controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genotype distribution between groups was contrasted by χ^2- test and the degree of genetic association was evaluated by 95% confidence interval (CI).

Results: Frequency of the variant AR C-106T allele was 13.9% (95% CI: 11.2%–16.6%) in the controls, which was significantly lower than that in the Japanese (18.4% in 712 individuals, P=0.0063), the Australians (37.9% in 240 individuals, P<0.0001) and the Brazilians (34.7% in 62 individuals, P< 0.0001). The frequency of AR C-106T allele was 11.7% (95% CI: 7.9%–15.5%) in the EH patients. No significant difference in the allele frequency was observed between the EH patients and the controls (P= 0.147).

Conclusion: There is obvious racial difference in the distribution of AR C-106T polymorphism. The polymorphism is not associated with the risk for EH.

KEY WORDS aldose reductase; polymorphism; essential hypertension

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醛糖还原酶基因 C-106T 多态性与原发性高血压易感性及其种族差异

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[摘要] **目的：**研究中国汉族人群中醛糖还原(AR)C-106T基因多态性的分布情况，比较其频率分布是否存在种族差异，并探讨该多态性与原发性高血压易感性的相关性。**方法：**应用聚合酶链反应－限制性片段长度多态性（polymerase chain reaction-restriction fragment length polymorphism，PCR-RFLP）的分析方法，在148位原发性高血压病人和137位正常受试者中，对AR基因C-106T多态性进行基因分型。频数分布的比较采用卡方检验，基因型与高血压的关联程度采用95%置信区间评估。**结果：**对照人群中AR C-106T等位的频率为13.9%（95% CI: 11.2%~16.6%），远低于日本人群（n=712, 18.4%, P=0.0063），澳大利亚人群（n=240, 37.9%, P<0.0001）和巴西人群（n=62, 34.7%, P<0.0001）。高血压病例和对照人群中AR-C106T等位基因的频率分别为15.9%（95% CI: 11.6%~20.0%）和11.7%（95% CI: 7.9%~15.5%），两组间基因型分布差异无统计学意义（P=0.147）。**结论：**醛糖还原酶AR基因C-106T多态性的频率分布具有明显的种族差异，该多态性与中国人群原发性高血压的发病风险不相关。

[关键词] 醛糖还原酶；基因多态性；原发性高血压

Aldose reductase (AR/ALR2), an enzyme sensitive to ROS, is a member of the NADPH-dependent aldo-keto reductase superfamily. The enzyme is monotonic and widely expressed in the body. The C-106T polymorphism located in the basal promoter region of AR gene was firstly identified by Kao et al in a group of Australian adolescents[1]. The susceptibility Z-2/C-106 and protective Z+2/T-106 promoter region polymorphisms were reported to influence the transcriptional level of AR by changing the efficiency of nuclear factor binding to CCAAT element[2]. Clinical relevance of the AR C-106T polymorphism has been extensively investigated, and the polymorphism is reported to be associated with the susceptibility to several diabetic mellitus related complications such as diabetic nephropathy, retinopathy and neuropathy[1, 3–6].

Previous studies have shown that the allele frequencies for AR C-106T are 18.4%, 37.9%, 28.0% and 34.7% in Japanese, Australia, Finland and Brazil populations, respectively [1, 7–10]. Our purpose of this study was to estimate the frequency of AR C-106T in Chinese Han people and compare its racial differences.

Recent reports indicated that an increase in AR expression in myocardial ischemic and vascular inflammation is associated with an increased reactive oxygen species (ROS) [11–13]. Inhibition of AR expression can ameliorate TNF-α-induced inflammatory response and redox-sensitive signaling pathways that play important roles in vascular smooth muscle cell (VSMC) proliferation and cardiac hypertrophy involved in several cardiovascular diseases including hypertension [14–17]. Meanwhile, our previous studies have also shown that AR inhibitors can improve the vascular remodeling in the spontaneous hypertensive rats (SHRs), a rat hypertensive model mimics essential hypertension in human. These indicated that AR may be involved in the development of essential hypertension, and functional polymorphisms in AR gene might be associated with risk for essential hypertension (EH) in human. Our study was also aimed to investigate association of AR C-106T polymorphism of with risk for EH in Chinese Han population.

1 Subjects and Methods

1.1 Subjects

From 2005 to 2008, a total of 148 patients onset of EH with blood pressure (BP)≥140/90 mmHg and 137 genetically unrelated age- and sex-matched healthy individuals with SBP ≤140 mmHg and/or DBP ≤90 mmHg (JNC VII) were recruited in the Heath Examine Center, Third Xiangya Hospital for the study. The blood pressures were measured in supine position by 2 trained observers for at least a 10-minute interval, with a calibrated mercury sphygmomanometer of appropriate adult cuff size according to a standard protocol recommended by the American Heart Association [18]. All subjects were Chinese Han individuals living in Hunan Province, middle-aged and were newly-diagnosed as EH. Individuals with diabetes mellitus, secondary hypertension, myocardial infarction, stroke, renal failure, drug abuse and other serious diseases were excluded. All individuals signed an informed consent before the study. Demographic information and clinical biochemical analysis results included age, sex, height, weight were recorded. None of the controls had any abnormalities according to their medical records, physical examinations, routine laboratory tests, or electrocardiography. For each person, 5 mL peripheral blood sample was drawn and collected into a sterile tube with 2% EDTA for DNA extraction. The protocol was approved by the Ethics Committee of Xiangya School of Medicine, Central South University, Hunan, China.

1.2 Genotyping of the AR C-106T polymorphism

Peripheral blood lymphocytes (PBLs) were isolated by centrifugation in a density gradient of Histopaque-1077 (15 min, 280 g). The pellet containing PBLs was resuspended in Tris-EDTA buffer at pH 8.0 for a production of 10^3 cells per well. Genomic DNA was extracted from PBLs with phenol-chloroform, followed by ethanol precipitation [19]. Genotyping analysis was carried out by using the PCR-RFLP assay. The PCR procedure for the AR gene was performed as described previously with minor modifications [20].

Primers for amplification the region of interest included the forward primer: 5'-CGCCGT TGTTGAGCAGGAGAC-3' and the reverse primer: 5'-TTCGCTTTCCCACCAGATAC-3'. The amplified DNA fragments including the C-106T polymorphic site (Figure 1) were digested with restriction enzymes *Bfa* I (TaRaRa Biotech, China) at 37 °C for 6 h. The different patterns produced by the digested fragments were visualized on 3.0% agarose gels stained with ethidium bromide. Following the amplifications, PCR products of 5% randomly selected samples were purified and sequenced to confirm the PCR-RFLP genotyping results.

The AR C-106T polymorphism gave rise to a *Bfa* I cleavage site. The PCR product of the AR C-106T allele contained a unique site for restriction by *Bfa* I. Two fragments of 234 and 92 bp were presented in CC homozygotes, 3 fragments of 175, 92 and 59 bp were presented in TT homozygotes, and 4 fragments of 234, 175, 92 and 59 bp, respectively, were observed for CT heterozygotes (Figure 2). For each randomly selected sample, the experiment of PCR-RFLP genotyping assay was repeated twice.

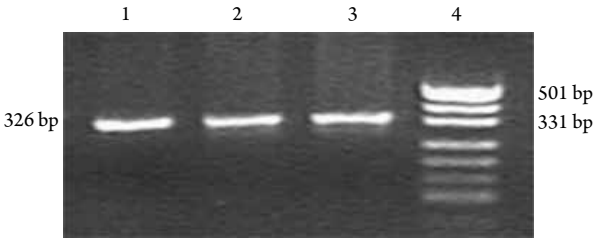

Figure 1 PCR amplified fragments for AR C-106T genotyping. Lane 1–3: Fragments of AR C-106T polymorphic sites. The length was 326 bp; Lane 4: DNA ladder marker.

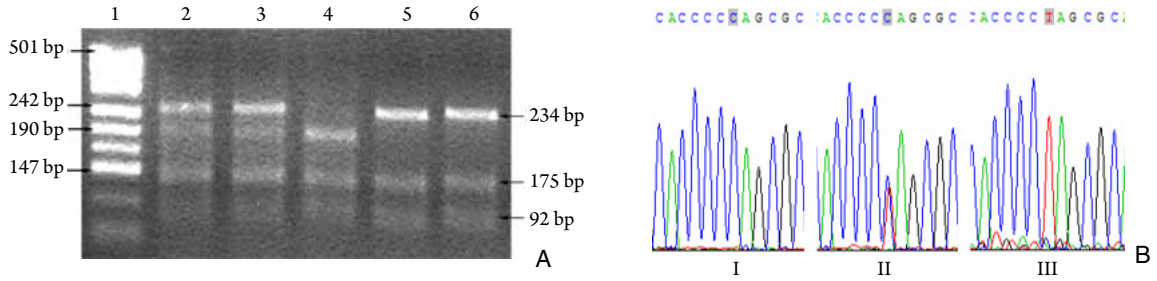

Figure 2 RFLP analysis of the AR C-106T polymorphism (A) and verification of genotyping by direct sequencing (B). Lane 1: DNA ladder marker; Lane 2–3: Heterozygous CT with 4 fragments of 234, 175, 92 and 59 bp; Lane 4: Homozygous CC individuals with 2 fragments of 234 and 92 bp; Lane 5–6: Homozygous TT with 3 fragments of 175, 92 and 59 bp. I: CC genotype; II: CT genotype; III: TT genotype.

1.3 Statistical analysis

Statistical analysis was performed by SPSS software (ver. 13.0 for Windows; SPSS, Chicago, USA). Comparison of difference in sex, age, height and weight (expressed as mean ± SD) were analyzed by χ^2 test or *t*-test. The χ^2 test was also used to compare the differences in allele frequencies of AR C-106T polymorphisms between groups. Hardy-Weinberg equilibrium (HWE) of genotypes was performed with HW Diagnostic software (Fox Chase Cancer Center, 1999). Two-tailed $P<0.05$ was considered to be statistically significant.

2 Results

The distribution of genotypes of AR C-106T polymorphism in 285 was in accordance with the HWE. The AR C-106T allele frequency was 13.9% (95%CI: 11.2%–16.6%) in the 285 Chinese Han individuals, whereas 18.4% (n=712, 95%CI: 16.4–20.5), 37.9% (n=240, 95%CI: 33.5–42.4), 28.0% (n=211, 95%CI: 3.7–32.5) and 34.7% (n=62, 95%CI: 26.4–43.7), respectively, in the population of Japan, Australia, Finland and Brazil (Table 1). The results from χ^2 test showed a significant difference between the Chinese Han population and the population in Japan (P=0.0063), Australia ($P<0.0001$) and Brazil ($P<0.0001$). Due to the wide confidence internal of the frequency in the Finnish population, the distributive

frequency of Finns at the point of 28.0 had no statistical significance.

Table 2 summarized the demographic data of the EH patients and the controls. There was no obvious difference in the sex distribution, age and height between the EH patients and the controls (P>0.05). Mean weight, DBP and SBP were significantly higher in the EH patients than the controls (P<0.05). Allele frequencies for the AR C-106T allele frequencies were 15.9% (95% CI: 11.6%–20.0%) and 11.7% (95% CI: 7.9%–15.5%), respectively, in the EH patients and the controls (Table 3). No significant difference in allele frequencies between the groups was observed (P=0.147).

Table 1 Comparison of AR C-106T allelic frequency in Chinese and other populations

Races	n	Allele Freq/No. (%)	95% CI	P
Chinese	285	79 (13.9)	11.2–16.6	
Japanese	712	262 (18.4)	16.4–20.5	0.0063*
Australians	240	182 (37.9)	33.5–42.4	<0.0001*
Finns	211	118 (28.0)	3.7–32.5	
Brazilians	62	43 (34.7)	26.4–43.7	<0.0001*

*P<0.05 vs Chinese.

Table 2 Clinical data of EH patients and controls

Groups	n	Sex (M/F)	Age/years	Height/cm	Weight/kg	DBP/mmHg	SBP/mmHg
Controls	137	86/51	46 ± 8	164 ± 8	64.0 ± 7.0	115 ± 8	81 ± 5
EH patients	148	91/57	47 ± 7	165 ±9	68.4 ± 7.3	149 ± 5	95 ± 4
P		0.823	0.263	0.321	<0.001	<0.001	<0.001

Table 3 Genotype and allele frequencies of AR C-106T polymorphism in EH patients and controls

Groups	CC Freq / No.(%)	CT Freq / No.(%)	TT Freq / No.(%)	T allele Freq/ No.(%)	T allele 95% CI/%
Controls	108 (78.8)	26 (19.0)	3 (2.2)	32 (15.9)	11.6 – 20.0
EH patients	104 (70.3)	41 (27.7)	3 (2.0)	47 (11.7)	7.9–15.5
P		0. 205		0.147	

3 Disscussion

In our study, we observe that the AR C-106T allele frequency is 13.9% (11.2%–16.6%) in Chinese Han populations. This is in agreement with that reported by Li et al [21]. However, the AR C-106T allele frequency in our population is markedly lower than that in the Japanese (n=712, 18.4%) [7], the Australians (n=240, 37.9%) [8] and the Brazilians (n=62, 34.7%) [10]. Because of somewhat wide confidence internal in the Finns [9], we observed no significant difference in allele frequency for the AR C-106 polymorphism between Chinese Han and the Finns. An increase in sample size for the latter may help to draw a confirmative conclusion.

Hypertension is a complex pathological disease that accompanied by up-regulated systemic inflammation [22–23], enhanced oxidative stress [24–25], and cardiac and vascular complications as well [26–28]. All these elements may lead to activation and increased expression of aldose reductase [29–32]. In turn, an increase in AR expression stimulates further elevation in blood pressure and facilitates the development of the disease, even leading to injury of hypertensive target organs[31]. We assume that AR should be closely interconnected with change of blood pressure.

This study investigated the association between AR C-106T polymorphism and risk for essential hypertension for the first time. However, we failed to find the associations between the polymorphism and disease susceptibility. The evaluation for the existence of a possible bias should be ascribed to 3 main reasons. Firstly, even though numerous studies have reported that AR C-106T polymorhpism is a risk factor for the early development of microalbuminuria[9, 32], diabetic microvascular complications[1–6] and stroke[34], the role of AR is not so important in the development of hypertension when compared with other complex environmental and genetic factors. Secondly, since Kao et al identified the AR C-106T polymorphism for the first time and found that the CC homozygotes and C allele were obviously higher in those with retinopathy when compared with controls (P=0.0035 and 0.005, respectively)[1]. The AR C-106T polymorphism may play more important role in the pathogenesis of microangiopathy[2, 3, 5] and macroangiopathy[7]. Small sample size may be the third cause we failed to find association between AR C-106T polymorphism and essential hypertension. Of course, involvement of the AR C-106T polymorphism in this multi-genetic disease needs further study with population with larger sample size.

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