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α-Adducin gene *G614T* polymorphisms in essential hypertension patients with high low density lipoprotein (LDL) levels

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***Background & objectives*: The association between α-adducin gene G614T polymorphism and essential hypertension (EH) is not clear. The present study was carried out to examine a possible association between α-adducin gene G614T mutation and essential hypertension in Chinese population.**

***Methods*: A total of 170 patients with essential hypertension (EH group) and 154 normotensive subjects (Control group) were genotyped for the cytoskeletal protein single nucleotide polymorphism G614T of the α-adducin gene by PCR-RFLP technique. Systolic blood pressure (SBP), diastolic blood pressure (DBP), body mass index (BMI), low density lipoprotein (LDL), high-density lipoprotein (HDL), high-sensitivity C-reactive protein (hs-CRP), left atrial diameter (LA DIA), left ventricular diameter (LV DIA) and other parameters were recorded in EH group.**

***Results:* There was significant association between EH and α-adducin genotypes ($P<0.05$). GT and TT genotypes in EH group had higher LDL levels as compared to GG carriers ($P<0.05$). The LDL concentration was significantly elevated in patients with GT and TT genotypes. The LDL levels also differed significantly in male patients with all the three genotypes.**

***Interpretation & conclusions:* A significant association was found between *ADD1* gene G614T polymorphism and EH in Chinese patients. Further studies need to be done to confirm these findings in a large sample.**

Key words α-adducin - essential hypertension - gene - LDL - polymorphisms

Essential hypertension (EH) is the most common cardiovascular disease worldwide. Epidemiological studies have shown that the incidence of hypertension is influenced by the genes, environmental factors and lifestyle of individuals. The occurrence of EH may be associated with a variety of gene mutations and variations including adducin gene (*ADD*)[1-3]. The α-adducin gene (*ADD1*) has been implicated in causing susceptibilty to hypertension, especially in relation to salt sensitivity[4]. *ADD1* gene polymorphism G614T (rs4961) is found to result in an increased enzymatic activity of the outer medulla Na+-K+-ATPase prior to the development of hypertension in the Milan hypertensive strain of rats (MHS)[5]. The G614T polymorphism results in the amino acid substitution of glycine by tryptophan (Gly460Trp) which is reported to

be associated with a salt sensitive form of hypertension patients[6]. There is no clear consensus on the α-adducin gene polymorphism (Gly460Trp) and risk of EH in Chinese population. Liu *et al*[7,8] conducted a meta-analysis in an effort to systematically explore the possible association and suggested that the Gly460Trp polymorphism might increase the risk of hypertension in Chinese populations, especially in Han Chinese.

The potential involvement of α-adducin Gly460Trp gene mutation in the pathogenesis of EH has been demonstrated in a few studies[9,10], but no definite conclusion could be drawn. The aim of the present study was, therefore, to analyze the possible association between G614T polymorphism of *ADD1* gene and hypertesion phenotype in patients with EH.

Material & Methods

Subjects: Patients with EH (n=170) and normotensive controls (n=154) were consecutively selected from hypertension outpatient clinic and medical center, respectively, affiliated to the hospital of Zhejiang Medical College, Hangzhou, PR China from February to August 2010. The normotensive controls were the people who received common physical examination in the hospital. All subjects gave written informed consent for participation in the study and the study protocol was approved by the medical ethics committees of the Zhejiang Medical College. The EH group consisted of 97 male and 73 female patients with mean age of 57.4 ± 24 yr. Subjects with a history of diabetes mellitus and renal failure were excluded. Patients on antihypertensive drugs were excluded. The control group consisted of 81 males and 73 females with mean age of 56.9 ± 9.0 yr. These individuals came for routine physical examination in the hospital and had no family history of EH. The blood samples (3 ml) were collected after overnight fasting at morning without stasis in EDTA vacutainers. The patients were selected according to the Seventh Report of Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (2003 JNC7) (*i.e.* systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mmHg were used as indication of hypertension). Patients with secondary hypertension, diabetes, abnormal liver and kidney function were excluded. All subjects were asked about smoking status. Body mass index (BMI) was calculated with the formula, weight (kg)/height (m^2).

Quantitative parameters: Plasma high-sensitivity C-reactive protein (hsCRP) levels were measured within 2-3 h after collection of blood samples by high sensitivity enzyme immunoassay (Dade-Behring, Marburg Germany). Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were assessed using Flex Reagent Cartridges (Dade-Behring, Marburg Germany).

Genotyping: Genomic DNA was extracted from peripheral blood using Blood Genome DNA Extraction Kit (TAKARA Biotechnology Co. Ltd., Dalian, Japan). The genotyping of G614T polymorphism of *ADD1* was done by PCR-RFLP technique[11]. DNA fragments were amplified in a total volume of 50 µl PCR reaction mixture containing 10 × buffer 5 µl, 2.5 mM dNTP 4 µl, forward primer (5'-ctcctttgctagtgacggtgattc-3') 0.5 µl, reverse primer (5'-gacttggcactgcttccattc**g**gc-3') 0.5 µl, double distilled water (DDW) 37.75 µl, Taq polymerase (TAKARA Biotechnology Co. Ltd., Japan) 0.25 µl and DNA 2 µl. Amplification was carried out under the following conditions: one cycle of 5 min at 95 °C, 35 cycles of 35 sec at 95 °C, 35 sec at 56 °C, and 35 sec at 72 °C, followed by 5 min at 72 °C. A mismatch, which introduces a Sau96I restriction site, was placed in the 3' region of reverse primer to enable genotyping via restriction digest (the mismatched nucleotide is underlined and in heavier version. Amplified products were digested with Sau96I enzyme (NBE Inc., US) at 37 °C for 16 h. All products were loaded onto 3 per cent MS-6 agarose (TAKARA Biotechnology Co. Ltd., Dalian), and electrophoresed. Bands were visualized and typed after GelRed staining (Biotium Company, US). The length of PCR amplification product with G614T was 147 bp. The Sau 96I restriction enzymes were used to distinguish 614G/T, resulting in 122 bp and 25 bp fragments in the presence of the G allele. The polymorphism analysis was performed by two persons independently in a blind fashion. More than 10 per cent of the samples were randomly selected for confirmation, and the results were 100 per cent concordant (Figure).

Statistical analyses: The SPSS 16.0 software (SPPS Inc. USA) was used in this study. The expected frequencies of the *ADD1* G614T genotypes were tested for the Hardy-Weinberg Equilibrium. Statistical differences for the distribution of genotypes G614T between EH and control groups were assessed by $\chi 2$ test. The relationships of the *ADD1* genotypes with the clinicopathologic parameters of patients were tested by t-test. Logistic regression analysis was performed to assess the independent effect of each risk factor on the occurrence of EH. The odds ratio (OR) was calculated

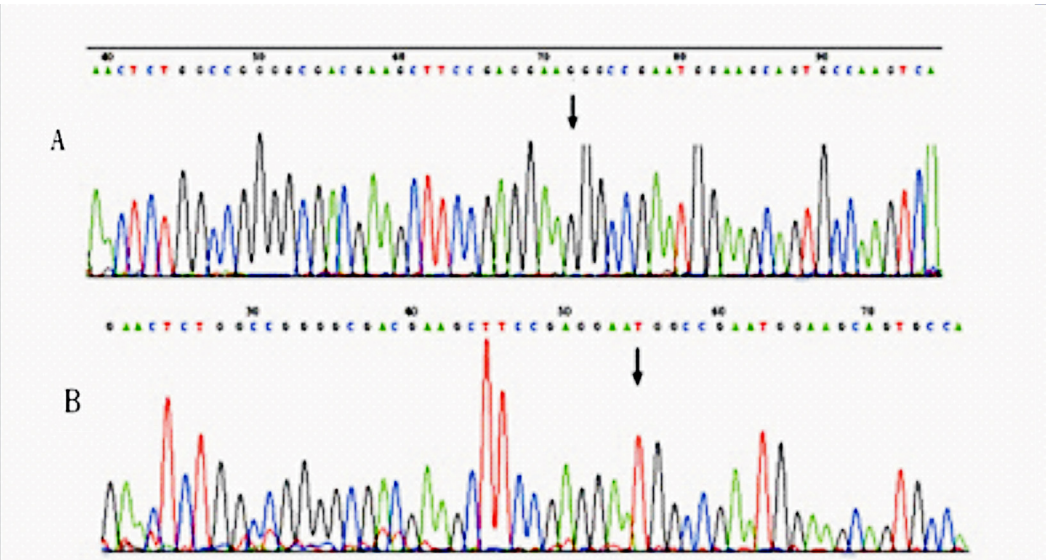

Fig. Chromatograms showing nucleotide sequence of *ADD1* gene G614T polymorphism. **(A)** Normal allele with G, **(B)** mutant allele with T. Substitution of the nucleotide indicated by arrows.

Table I. Clinical characteristics of essential hypertensive (EH) and normotensive groups

	EH	Control
N	170	154
Men (%)	57.1	52.6
Age (yr)	57.4±24.0	56.9±9.0
SBP (mmHg)	157.0±10.8*	118.2±13.2
DBP (mmHg)	82.7±15.2*	72.8±7.7

Data expressed as mean ± SD or per cent. *P<0.05, compared with the control

as estimators of relative risk, together with their 95% confidence intervals (95% CI). The difference in the clinicopathologic parameters according to *ADD1* G614T genotype distributions was compared using ANOVA test.

Results

No significant differences were observed with respect to age, sex, smoking status, serum HDL, CRP levels between hypertensive subjects and the controls. However, the EH group had a significantly (P<.05) higher blood pressure (Table I), BMI and the serum LDL concentration compared to the control group. Table II shows the genotype distributions and the allele frequencies of *ADD1* G614T polymorphism in the two groups. A significant difference in genotype distributions between hypertensives and controls was noted with the observed power of 0.899. The TT genotype was significantly (P<.05) higher in hypertensives as compared with controls (TT: 31.18 vs. 16.88%). From the "Genetic Power Calculator", the genotypic risk for TT was 12.27 per cent. For G and T allele frequencies, there was a significant (P<.05) difference between hypertensives and controls as well (G: 41.76 vs. 57.47%, T: 58.24 vs. 42.53%). The frequencies of the genotypes were significantly different between the patient and control groups (P<0.05). Both genotypes and alleles frequencies in control and EH groups were in Hardy-Weinberg equilibrium. By univariate analysis, it was found that EH was influenced by BMI, LDL and *ADD1* gene 614T allele. By multivariate logistic

Table II. Allele frequencies of α-adducin gene (*ADD1*) G614T polymorphism in normotensive and hypertensive groups

n		Genotype frequency			Allele frequency	
		GG	GT	TT	G	T
EH	170	25 (14.71)	92 (54.12)	53 (31.18)	142 (41.76)	198 (58.24)
Control	154	49 (31.82)	79 (57.29)	26 (16.88)	177 (57.47)	131 (42.53)
P value			<0.05		<0.05	

Values in parentheses are percentages

Table III. Clinical characteristics of each genotype in essential hypertensive groups

	EH		
	GG (n=25)	GT (n=92)	TT (n=53)
SBP (mmHg)	158.1±11.0	155.5±10.2	159.3±11.2
DBP (mmHg)	84.4±14.1	81.8±16.3	83.4±13.4
BMI (kg/m^2)	30.0±3.0	26.6±3.1	26.3±2.7
Smoking (%)	48	57	58
LDL (mg/l)	73.5±19.6*,**	98.6±23.3	95.0±23.7
HDL (mg/l)	40.4±10.2	40.1±10.0	43.0±8.0
hsCRP (mg/l)	21.1±10.9	17.9±10.4	17.5±12.0
LV DIA (mm)	52.5±5.2	52.9±7.4	52.9±4.9
LA DIA (mm)	34.0±5.5	35.1±5.5	34.2±4.3

The concentration of plasma LDL differed significantly between the three genotypes of EH groups, while HDL, LA DIA, LV DIA and other indicators did not differ between the three genotypes. Though lack of statistical significance, the plasma CRP concentration was lower in T allele carriers than GG carriers in EH group. $^{*}P<0.05$, compared with GT; $^{**}P<0.05$, compared with TT
SBP, systolic blood pressure; DBP, diastolic blood pressure ; BMI, body mass index; LDL, low density lipoprotein ; HDL, high density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LV DIA, left atrial diameter; LA DIA, left ventricular diameter

regression, T allele (OR=2.217, 95% CI: 1.243–3.953, P=0.007<0.05/3=0.017) was the independent risk factor of essential hypertension (Table III).

The demographic and clinical characteristics in EH group were studied according to the *ADD1* G614T genotype distributions. It was found that the concentration of plasma LDL differed significantly between the three genotypes in EH group (Table III). But the level of LDL was not associated with *ADD1* gene G614T polymorphism in the control group (data not shown). When subdivided according to gender, no significant association was observed with respect to clinical characteristics in female patients, while LDL was significantly elevated in the male patients (P<0.05). Though there was lack of statistical significance, the plasma CRP concentration was lower in patients who were T allele carriers than in those with GG genotype.

Discussion

ADD1 G614T polymorphism plays a potential biological role in the development of high blood pressure[12]. Several previous studies revealed that this variant was a likely candidate for studying association with hypertension status[13-16]. In this study, there was a significant difference in α-adducin genotype between EH and control groups. The levels of LDL were significantly increased in persons with GT and TT genotypes in EH group as compared with GG genotypes.

The frequency of α-adducin gene G614T varies in different populations. In our study, the frequency of α-adducin gene 614T in south China was 42.53 per cent in normotensives and 58.24 per cent in hypertensives. The frequency of α-adducin gene 614T allele in hypertensives and controls were 46 and 48 per cent in Chinese[17], 54 and 60 per cent in Japanese[17], 59 and 61 per cent in Koreans[18] and 53 and 33 per cent in Americans[14].

Several studies have demonstrated the potential involvement of α-adducin in the pathogenesis of EH, but no definite conclusion could be drawn (Table IV). Factors like ethnic diversity, sample sizes are expected to be the cause for these inconclusive results found. A sample of 904 African Americans (from Jackson, Mississippi) was examined for α- adducin gene 614T association with hypertension[20]. The results showed that African Americans not only have a higher prevalence of hypertension, but also the condition strikes at an early age, with greater severity, often ending in death when compared with whites in the United States. No association was found between hypertension status and the *ADD1* G614T polymorphism in American Blacks[23], white population of USA, Australia[21-24]. A case-control study conducted in a large population from Sassari, Italy, did not find any association of the α-adducin T allele with hypertensives while the study in a large population from Milan, Italy confirmed a positive association[25]. No significant association was found in a well characterized Japanese population[22] and Korean population that was also Asian descent[19]. G614T polymorphism in Chinese Han population of Shang Hai also reported absence of association with

Table IV. Frequency of α-adducin gene (*ADD1*) G614T varies in different populations

	Races	n		614T (frequency, %)		*P*
		EH	Control	EH	Control	
Melander el al[14]	Scandinavian	294	265	17	21.7	<0.05
Huang *et al*[15]	Han Chinese	256	495	35.2	27.4	<0.05
He *et al*[17]	Chinese	138	121	48	46	>0.05
Shin *et al*[19]	Korea	321	582	61.1	59.4	>0.05
Larson *et al*[20]	Afican American	472	432	6.8	7	>0.05
Wang *et al*[21]	Australian	112	196	23	24	>0.05
Kato *et al*[22]	Caucasian	223	159	56	60	>0.05
Present study	Chinese	170	154	58.24	42.53	<0.05

Allele-specific oligonucleotide hybridization; *P* value was the result of association study

hypertension[26]. Adjusted for the conventional risk factors of hypertension, alpha-adducin polymorphism has been shown to play an independent role on systolic blood pressure in Indians living in Car Nicobar Island[16]. Our study showed that there was significant association between G614T substitution of *ADD1* gene and hypertesion phenotype of EH patients in South China.

In our study, the concentration of plasma LDL was higher in hypertensives carrying at least one 614T allele than in GG homozygotes. Furthermore, no association of LDL concentration and the mutation was observed in female hypertensives. Males with 614T allele had higher LDL, suggesting risk for cardiovascular diseases. Castejon *et al*[27] reported that the concentration of plasma LDL was significantly different in the GG and GT healthy groups. However, because of the small sample size (n=90), TT homozygotes were not detected. In our study, TT homozygotes were detected and EH patients with T allel had higher plasma LDL level. The mechanism by which *ADD* polymorphism influences LDL levels is not clear and further study is needed to investigate underlying factors in detail.

In conclusion, our results indicate towards genetic association between α-adducin gene G460T polymorphism and hypertension. Further studies need to be done on the association of this polymorphism with hypertension in different ethnic groups with larger samples.

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