



## Polymorphisms of +2836 G>A in the *apoE* gene are strongly associated with the susceptibility to essential hypertension in the Chinese Hui population

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Genet. Mol. Res. 13 (1): 1212-1219 (2014)

Received December 19, 2012

Accepted July 23, 2013

Published February 27, 2014

DOI <http://dx.doi.org/10.4238/2014.February.27.6>

**ABSTRACT.** In the present study, the correlation of polymorphisms of the apolipoprotein E (*apoE*) gene with the susceptibility of essential hypertension (EH) was investigated. Single nucleotide polymorphisms of the *apoE* gene at the -491 A>T, +969 C>G, and +2836 G>A sites were determined in 221 non-EH individuals and 109 subjects with EH of Chinese Hui ethnicity using polymerase chain reaction-restriction fragment length polymorphism analysis. The results showed that neither the genotypic frequency nor the allelic frequency at the -491 A>T and +969 C>G sites exhibited a statistically significant difference between these two groups ( $P > 0.05$ ). However, a significant difference was observed in genotypic frequency and allelic frequency at the +2836 G>A site between EH patients and non-EH individuals ( $P < 0.01$ ). In addition, a significantly higher frequency of the A allele at the +2836 G>A site was also detected in EH patients (83%) compared with controls (47.5%) ( $P < 0.01$ ; OR = 4.82, 95%CI = 3.25-7.17);

in contrast, the frequency of the G allele at the +2836 G>A site was significantly lower (17%) in the patient group in comparison with the non-EH cohorts (52.5%) ( $P < 0.01$ ; OR = 0.21, 95%CI = 0.14-0.31). These results suggest that the polymorphism at the +2836 G>A site in the *apoE* gene is strongly correlated with the susceptibility to EH in the Chinese Hui ethnic population.

**Key words:** Essential hypertension; Single nucleotide polymorphism; Apolipoprotein E; Chinese Hui population

## INTRODUCTION

Apolipoprotein E (ApoE), along with ApoA, ApoB, ApoC, ApoD, ApoM, ApoH, ApoJ, and ApoL, are members of the apolipoprotein gene family (Dawar et al., 2010). The *apoE* gene consists of 4 exons and 3 introns, spanning 3597 nucleotides, and encoding a 299-amino acid polypeptide. This gene is located on chromosome 19q13.2 and is closely linked to the apoC-I/C-II gene complex (Scott et al., 1985). ApoE is mainly produced in the liver, but other organs and tissues such as the brain, spleen, kidneys, gonads, adrenals, and macrophages also produce this protein (Mahley, 1988). Accumulating evidence reveals that the polymorphism of the *apoE* gene is genetically associated with many diseases, including essential hypertension (EH), coronary artery disease, polycystic ovary syndrome, Alzheimer's disease, psoriasis, vascular dementia, gallbladder stone disease, and cerebrovascular disorders (Karpouzis et al., 2009; Chaudhary et al., 2012; Liu et al., 2012a; Xue et al., 2012; Yin et al., 2012; Zhou et al., 2012).

Hypertension is a major risk factor for cardiovascular disease, stroke, and end-stage renal disease and prevails globally; thus, the prevention of hypertension remains an important public health goal worldwide (Izawa et al., 2003). EH, also known as primary hypertension, is a common disease influenced by polygenic and multifactorial disorders that are likely due to interactions between the genetic constitution of populations as well as environmental factors (Li, 2012), which is one of the most common complex diseases, accounting for 95% of all cases of hypertension (Niu et al., 2007a). To date, approximately 1 billion people are suffering from EH worldwide, and the current occurrence of EH in China is about 11.8%. In addition, approximately 2 million new cases of cerebrovascular diseases are added annually in China, 60% of which are associated with EH (data from the Ministry of Health of the People's Republic of China) (Li and Liu, 2012). Therefore, discovering a novel EH-associated gene may provide insight into the pathogenesis of EH and help to develop novel therapeutic strategy for EH.

Previous studies have demonstrated that genetic variations and polymorphic associations of TIM genes and susceptibility to rheumatoid arthritis in the Chinese Hui minority ethnic population (Xu et al., 2012a,b). The association between the polymorphisms of the *apoE* gene with the susceptibility to EH, together with the genetic variations of the *apoE* gene among different ethnic populations (Mahfouz et al., 2006; Niu et al., 2007b), imply that genetic polymorphism of the *apoE* gene may be correlated with the susceptibility to EH in the Chinese Hui population. The objective of this study was to explore the association of the *apoE* gene and the susceptibility to EH in this ethnic population by examining the polymorphisms of the -491 A>T, +969 C>G, and +2836 G>A loci of the *apoE* gene.

## MATERIAL AND METHODS

### Subjects

Blood samples were collected from 109 EH patients and 221 non-EH control individuals of the Chinese Hui population. All individuals were living in the Ningxia Hui Autonomous Region of China. There was no genetic relationship among these individuals. All the samples were collected under informed consent. The World Health Organization criteria for the classification of EH were used to diagnose patients with EH (Gan et al., 2012). The non-EH controls were recruited from the general Hui population and had undergone comprehensive medical screening at the Affiliated Hospital of Ningxia Medical University. All subjects were included in this study based on 2 criteria: 1) of purely Hui descent for at least 3 generations and 2) individual ancestors lived in the Ningxia region for at least 3 generations.

### Single nucleotide polymorphism (SNP) analysis

Genomic DNA was extracted from white blood cells. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and nested-PCR were performed on 3 SNPs, the -491 A>T, +969 C>G and +2836 G>A sites, of the human *apoE* gene. PCR was performed in a 25- $\mu$ L reaction volume using 200 ng genomic DNA. The DNA was amplified using 35 cycles at 94°C for 30 s, 55°-65°C for 45 s (Table 1), and 72°C for 45 s, with a final extension at 72°C for 5 min with a BioRad MyCycler thermal cycler (BioRad Laboratories, Hercules, CA, USA). Genotyping of the -491 G>T, +969 C>G and +2836 G>A SNPs of the *apoE* gene was performed by nested-PCR as previously reported (Roks et al., 1998). PCR-RFLP analysis was employed for genotyping of these polymorphic sites as described previously. The primer sets used for PCR as well as restriction endonucleases used for digestion are listed in Tables 1 and 2, respectively. For PCR-RFLP analysis, the PCR products were purified using a PCR purification kit, followed by digestion with restriction endonucleases *DraI*, *BspLI*, and *ScaI* (Table 2). The digested PCR products were separated on non-denatured PAGE, and the gels were stained by silver nitrate (Perry and Peyvandi, 1999). The size of the PCR products and their corresponding digested products are listed in Table 2. The PCR kit, PCR purification kit, and restriction endonucleases were purchased from Takara Biologicals (Tokyo, Japan).

**Table 1.** Sequences of primers for amplification of the *apoE* gene in this study.

Sites	Primers sequence	Annealing temperature (°C)	Size of PCR products (bp)
-491 A>T	P1: 5'-CAAGTCACACAGCTGGCAAC-3'	55	227
	P2: 5'-TCCAATCGACGGCTAGCTACC-3'		
969 C>G	P1: 5'-TGAGAAGCGCAGTCGGGGGCA-3'	65	104
	P2: 5'-AGGTCCAAGTCCCCTGCTGCT-3'		
2836 G>A	P1: 5'-GCTTTCCAAGTGATTAACCGACT-3'	55	197
	P2: 5'-CTCCGGCTCTGTCTCCACCGGAGCT-3'		

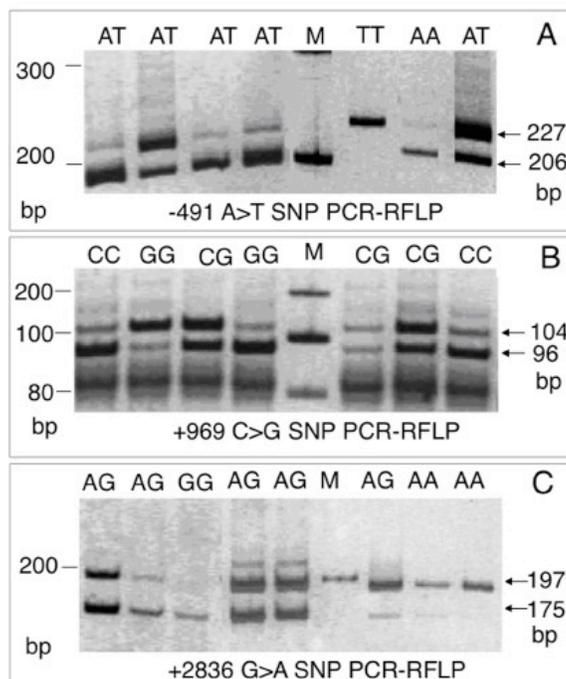
### Statistical analysis

The genotype and allele carrier frequencies were defined as the percentage of indi-

viduals carrying the genotype and allele, respectively, of the total number of individuals. The  $\chi^2$  test and Fisher exact test of SPSS 17.0 for Windows (SPSS, Chicago, IL, USA) were used to test for deviation from the Hardy-Weinberg equilibrium and to compare to the frequency of discrete variables between EH patients and control individuals. P values less than 0.01 were considered to be statistically significant.

## RESULTS

The 3 SNPs of the *apoE* gene and the polymorphic associations with susceptibilities to various diseases in different populations have been demonstrated (Ahmed et al., 1999). To determine if these SNPs are also associated with susceptibility to EH in the Chinese Hui population, we analyzed the polymorphism of the *apoE* gene using SNPs of -491 G>T, +969 C>G, and +2836 G>A in 109 EH patients and 221 non-EH control individuals from the Hui population using PCR-RFLP. Following *DraI*, *BspLI*, and *SacI* digestion of PCR products of these 3 polymorphic sites, respectively, all three genotypes were determined for each SNP (Figure 1 and Table 2). The genotypes of SNP -491 G>T were TT, GT, and GG; the genotypes of SNP +969 C>G comprised GG, GC, and CC; and the genotypes detected for the +2836 G>A SNP were AG, GG, and AA (Figure 1 and Table 2). The characteristics of the genotype and the allelic frequencies of the 3 SNPs of the *apoE* gene are shown in Table 3.



**Figure 1.** Genotype analyzed by polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP). The PCR amplified products were digested with: **A.** *DraI* (for -491 G>T SNP), **B.** *BspLI* (for +969 C>G SNP), and **C.** *SacI* (for +2836 G>A SNP) before they were resolved using SDS-PAGE and visualized by silver nitrate. Lane M shows DNA molecular ladders. All other lanes show the corresponding genotypes indicated at the top of each picture.

**Table 2.** Determination of genotype by PCR-RFLP assay.

SNPs	Restricted enzymes	Genotype	Digested fragments (bp)
-491 A>T	<i>DraI</i>	TT	227
		AA	206, 21
		AT	227, 206, 21
969 C>G	<i>BspLI</i>	GG	104, 38, 21
		CC	95, 38, 21, 9
		CG	104, 95, 38, 21, 9
2836 G>A	<i>SacI</i>	AA	197,
		GG	175, 22
		AG	197, 175, 22

**Table 3.** Distributions of genotypic and allelic frequencies of the three SNPs in the *apoE* gene of essential hypertension (EH) patients and non-EH controls.

SNPs	Genotypes	Control [N (%)]	EH [N (%)]	$\chi^2$	P	OR (95%CI)
-491 A>T	TT	1 (0.5)	0 (0)	1.3	>0.05	T
	AA	188 (85.1)	97 (89.0)			A
	TA	32 (14.5)	12 (11.0)	1.1	>0.05	0.70 (0.35-1.38)
	T	34 (7.7)	12 (5.5)			1.43 (0.73-2.83)
969 C>G	A	408 (92.3)	206 (94.5)	1.0	>0.05	
	GG	75 (34.0)	33 (30.3)			
	CC	55 (25.0)	25 (22.9)	0.002	>0.05	0.99 (0.72-1.38)
	CG	91 (41.0)	51 (46.8)			1.01 (0.73-1.39)
	G	240 (54.5)	118 (53.7)			
2836 G>A	C	202 (45.5)	100 (46.3)	93.3	<0.01	
	AA	39 (17.6)	78 (71.6)			
	GG	41 (18.5)	6 (5.5)	66.6	<0.01	4.82 (3.25-7.17)
	AG	141 (64.0)	24 (22.4)			0.21 (0.14-0.31)
	A	219 (47.5)	180 (83.0)			
G	223 (52.5)	38 (17.0)				

Polymorphic analysis of the -491 G>T and +969 C>G SNPs showed no statistical difference in the genotypic and allelic frequencies between the EH patients and the controls, as determined by a Hardy-Weinberg equilibrium analysis ( $P > 0.05$ ) (Table 3). However, a statistically significant difference in the genotypic frequency of the +2836 G>A SNP was detected between the EH patients and the non-EH individuals ( $P < 0.01$ ) (Table 3). The relative frequencies of AA, AG, and GG were 71.6, 22.4, and 5.5% in EH patients; 17.6, 64.0, and 18.5% in non-EH individuals. The allelic frequency at the +2836 G>A polymorphic site was also significantly different between the EH patients and the controls (Table 3). The frequency of the A allele at this site was significantly higher (83.0%) in the EH patient group relative to the control group (47.5%) ( $P < 0.01$ ; OR = 4.82, 95%CI = 3.25-7.17); the frequency of the G allele at the +2836 G>A site was significantly lower (17.0%) in the patient group compared with the control group (52.5%) ( $P < 0.01$ ; OR = 0.21, 95%CI = 0.14-0.31). These findings suggested that the polymorphism at the +2836 G>A site in the *apoE* gene is strongly correlated with the susceptibility to EH in the Chinese Hui ethnic population, in which individuals with the G allele were less likely to have EH, while those with the A allele might be at risk for EH in the Chinese Hui ethnicity.

## DISCUSSION

We studied the correlation between the *apoE* gene and susceptibility to EH in the Chinese Hui population by determining the polymorphism of 3 SNPs, -491 A>T, +969 C>G and

+2836 G>A. The results demonstrated that there was no significant difference in the genotype and allele distributions of the -491 A>T and +969 C>G SNPs between the EH patients and non-EH controls. Interestingly, the distributions of the genotypes and alleles of the +2836 G>A SNP were significantly different between patients with EH and non-EH cohorts in the Hui population living in the Ningxia area ( $P < 0.01$ ), suggesting that the polymorphism at +2836 G>A of the *apoE* gene was strongly associated with the susceptibility to EH, where the A allele of the *apoE* gene may be a risk factor for EH in Hui individuals and the G allele may serve as a protective factor for EH. This genetic variation at the +2836 G>A site of the *apoE* gene was first described by Matsunaga et al. (1995), who demonstrated that the G>A mutation resulted in the change of a glutamic acid to a lysine residue. Additionally, polymorphisms of the *apoE* gene have also been correlated with the plasma lipid level (Utermann et al., 1979) and susceptibility to hypertension in a variety of ethnic groups (Liu et al., 2012b), although inconsistent results have been reported.

The increasing incidence of hypertension in China, and in other countries worldwide, suggests that hypertension has complex multifactorial causes. The risk factors of hypertension can be from the daily diet, obesity, contraceptive use, lifestyle, obstructive sleep apnea syndrome, as well as genetic variants (Ramu et al., 2009; Zhu et al., 2009; Chen et al., 2010; Xi et al., 2012a). Furthermore, recent molecular epidemiology studies revealed that certain genetic variants are associated with susceptibility to hypertension. In particular, polymorphisms of genes, including the leptin receptor, angiotensinogen, cytochrome P450, vascular endothelial growth factor, adrenoceptor, ApoB, bradykinin beta 2 receptor, and apolipoprotein CIII, have been associated with the susceptibility to this disease (Charita et al., 2012; Fowdar et al., 2012; Gu et al., 2012; Hamedian et al., 2012; Li and Liu, 2012; Shin et al., 2012; Tang et al., 2012; Xi et al., 2012b). Similar to variants of other genes in the apolipoprotein family, *apoE* gene polymorphisms may also contribute to the occurrence of hypertension in various populations. Indeed, data from this study demonstrated a strong correlation between the +2836 G>A polymorphism and susceptibility to EH in the Chinese Hui population.

In summary, the results presented in this study demonstrated a strong correlation between +2836 G>A polymorphisms of the *apoE* gene and susceptibility to EH in the Chinese Hui population, in which individuals with the A allele at the +2836 G>A site of the *apoE* gene may be susceptible to EH, while those who have the G allele may have less likelihood to contract this disease. These findings indicate that this SNP may be a potentially useful genetic marker for EH in this ethnic population. However, haplotype analysis may be necessary to discover the accurate genetic marker for EH. Moreover, studies with different populations are also required to elucidate the relationship between polymorphisms of the *apoE* gene and EH as well as the molecular mechanisms of the ApoE protein in the pathogenesis of EH.

## ACKNOWLEDGMENTS

Research supported by the Natural Science Foundation of Ningxia Hui Autonomous Region of China (Grant #NZ1120) and the Scientific Research for Colleges and Universities in Ningxia Hui Autonomous Region (2011).

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