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# Haplotype-based case-control study of the human AGTR1 gene and essential hypertension in Han Chinese subjects

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**Abstract**

**Objectives:** Essential hypertension is considered to be a multifactorial trait resulting from the combined influence of environmental and genetic determinants. The aim of the study is to assess the association between the human AGTR1 gene and essential hypertension (EH) using a haplotype-based case-control study in Han Chinese subjects.

**Design and methods:** Seven tag SNPs and the A1166C polymorphism of the AGTR1 gene were genotyped in 510 hypertension subjects and 510 normotensive subjects using PCR-RFLP method.

**Results:** Single SNP analyses indicated that the rs12695895 was significantly associated with hypertension, adjusted for covariates. Compared with the other haplotypes, Hap4 (AGGACTT) which carry the susceptible rs12695895 A allele was found to significantly increase the risk of EH with odds ratios equal to 1.84 ($p=0.0002$).

**Conclusions:** The present results indicate that rs12695895 might be a genetic marker for EH and Hap4 (AGGACTT) was associated with hypertension in Han Chinese population.


*Keywords:* Haplotypes; AGTR1; Essential hypertension; Association study; Single-nucleotide polymorphism; tag SNP

## Introduction

Essential hypertension (EH) is a major risk factor for many common causes of morbidity and mortality including stroke, myocardial infarction, congestive heart failure, and end-stage renal disease [1]. The etiology and pathogenesis of EH are likely composed of a multi-factorial disorder that results from the inheritance of several susceptibility genes as well as from multiple environmental determinants [2]. Because of the central role of the renin–angiotensin system (RAS) in the blood pressure regulation, genes of the RAS remain important.

Angiotensin II is a potent vasopressor hormone and a primary regulator of aldosterone secretion. It is an important factor controlling blood pressure and volume in the cardiovascular system [3]. Angiotensin II exerts its effects *via* two structurally distinct receptor subtypes: the angiotensin II type I receptor (AGTR1 or AT1R) and the angiotensin II type II receptor (AGTR2). The AGTR1 receptor belongs to the G-protein coupled receptor super-family with 7 trans-membrane domains [4]. It mediates most of the known effects of angiotensin II, i.e., vasoconstriction, stimulation of $Na^+$ re-absorption and aldosterone biosynthesis, induction of cellular growth and hypertrophy, and facilitation of sympathetic neurotransmission [5]. The AGTR1 gene is localized in chromosome 3q21–q25, which consists of five exons and four introns [6], and spans approximately 45.1 kb. Exon sizes

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range from 58 bp to 2013 bp. Exon 5 is the largest and the only coding exon, while the first four exons encode a 5′ untranslated region [7].

AGTR1 plays an integral role in blood pressure control, and is implicated in the pathogenesis of hypertension [8]. Association between the AGTR1 gene polymorphisms and essential hypertension, including the A1166C polymorphism (rs5186), has been widely studied. However, controversial results have been reported [8-12]. The causes of these discrepancies are multiple. The most important cause is that hypertension is a polygenic disorder, but not a monogenic trait. One gene may be responsible for the disorder in one population, but not necessarily in another population. Other possible reasons include a spurious association caused by population stratification, epistatic gene-gene interactions or a specific multilocus haplotype, rather than any of the single loci that define the haplotype, is more significant in determining the association [13]. The inconsistencies of gene association studies by a single-nucleotide polymorphism (SNP) analysis observed in different populations demonstrate the challenges of dissecting complex multifactorial traits like essential hypertension. One possible way to overcome the limitation of previous association studies is to look for susceptible haplotypes of the AGTR1 gene for hypertension rather than focusing on single polymorphism.

The aim of the present work was to assess the role of AGTR1 genotypes and haplotypes as potential risk factors for hypertension in Han Chinese population.

## Materials and methods

### *Subjects*

The1020 participants were randomly recruited from the outpatients clinic and people undergoing a medical check-up in the Third People's Hospital of Yunnan Province and Kunming Yan'an Hospital. In this case-control study, an essential hypertensive (EH) group was composed of 510 patients diagnosed with EH was defined as blood pressure (BP) ≥ 140/90 mm Hg or current treatment for hypertension with antihypertensive medication. Participants with diabetes mellitus, secondary hypertension, myocardial infarction, cerebrovascular accident or other serious diseases were excluded. Normotensive group (NT) was composed of 510 subjects with blood pressure <140/90 mm Hg and without history of hypertension who were chosen based on their comparable sex, age with their hypertensive counterparts. Table 2 showed the characteristics of the NT and EH groups.

### *SNP identification and genotyping*

The AGTR1 common SNPs (minor allele frequency [MAF] > 5%) were searched from the Han Chinese data set of the International HapMap Consortium (http://www.hapmap.org/index.html.ja). The sets tag SNPs was selected to predict the remaining common SNPs with a $r^2 \geq 0.8$ using the Haploview 4.1 software (http://www.broad.mit.edu/mpg/haploview). According to the criteria, 7 tag SNPs (rs10935724, rs2638360, rs3772616, rs12695895, rs385338, rs12695908, and rs6801836) of the AGTR1 gene were selected. rs10935724 is located in intron 1; rs2638360, rs3772616 and rs12695895 are located in intron 2; rs385338, rs12695908 and rs6801836 are located in intron3. In addition, rs5186 (A1166C) which locates in the 3′-UTR region was also selected in this study. The AGTR1 gene structure and relative position of these 8 SNPs are shown in Fig. 1.

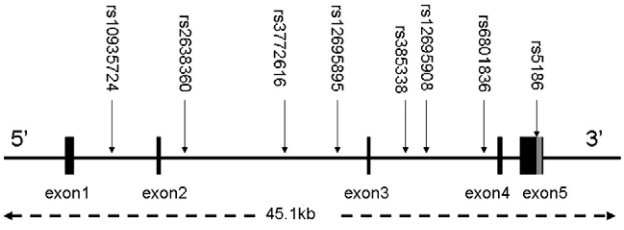


Fig. 1. The AGTR1 gene structure and relative position of 8 SNPs. Black boxes indicate exons, lines indicate introns, and gray box indicate 3′-UTR.

DNA was isolated from peripheral leukocytes with a standard phenol-chloroform method, and stored at −70 °C. All selected SNPs were genotyped in all 1020 subjects by polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP). The primers, length of PCR products, related restriction endonuclease, as well as digested bands are shown in Table 1. Each 15-μL reaction system consisted of approximately 25 ng of genomic DNA, 0.1 μM each primer, 200 μM dNTPs, 1.0 unit of Taq polymerase (TaKaRa Biotechnology Co. Ltd., Japan) and 1.5 μL 10×PCR buffer (TaKaRa). Samples were subjected to denaturation at 95 °C for 5 min, followed by 37 cycles of 95 °C for 30 s, annealing at 54 °C (for rs10935724, rs2638360 and rs12695895), 56 °C (for rs385338, rs12695908 and rs6801836), 57 °C(for rs5186)or 63 °C (for rs3772616) for 30 s, then extension at 72 °C for 30 s, and a final step at 72 °C for 10 min. PCR products were digested with 20 unit restriction enzymes following the manufacturer's instructions. Digested fragments were separated by electrophoresis on 3% agarose gel and identified by ethidium bromides staining. About 10% of the samples were random selected to perform repeat assays as genotyping quality control.

### *Statistical analysis*

All continuous variables are shown as means ± SD. Hardy–Weinberg equilibrium was assessed by performing a $\chi^2$ analysis. The genotypic and allelic frequency between cases and controls were compared by a two-sided Fisher exact test. Logistic regression analysis was used to estimate the independent effect of each genetic variant on the risk of EH. The statistical analyses were performed with the statistical software package SPSS 11.0 (SPSS, Chicago, IL).

Lewontin's D' (|D'|) and $r^2$ between each pair of genetic markers were calculated using SHEsis Software (http://analysis.bio-x.cn/myAnalysis.php). Based on the genotyped data of the genetic variations, the AGTR1 haplotypes and their estimated frequencies were defined using the Full-Precise-Iteration (FPI) algorithm [14]. In this haplotype-based case-control study, haplotypes with a frequency of <0.01 were excluded. The