Original Article

Relationship of ACE2350 G/A and chymase genetic polymorphisms with left ventricular hypertrophy in Chinese essential hypertension patients

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Abstract: 761 patients with essential hypertension (EH) were divided into left ventricular hypertrophy (LVH) (EH with LVH) group and EH (EH without LVH) group, 682 subjects undergoing physical examination served as control group. The gene polymorphisms of ACE2350 G/A and chymase were detected. Results showed that the distribution of ACE 2350 G/A genotypes (GG, GA, AA) was 22.9%, 51.4% and 25.7% for LVH group, 30.1%, 48.0% and 21.9% for EH group, and 34.8%, 51.4% and 13.9% for control group. The derived allele frequencies for the G and A allele were 48.6% and 51.4% in LVH group, 53.9% and 46.1% in EH group, and 60.2% and 39.5% in control group. The frequency of AA genotype and A allele of ACE2350 gene was significantly higher in LVH group than in control group (P<0.05). The distribution of CMA/B genotypes (GG, GA, AA) was 62.7%, 28.6% and 8.6% for LVH group, 57.0%, 28.1%, 14.9% for EH group, and 52.4%, 34.8%, and 12.8% for control group. The derived allele frequencies for the G and A allele were 77.1% and 22.9% in LVH group, 71.1% and 28.9% in EH group, and 69.9% and 30.1% in control group. No significant difference was found in the frequency of G and A allele of CMA/B gene between any two groups. In conclusion, ACE2350 gene polymorphism is closely related with LVH. AA genotype increases the risk of LVH, and A allele is the risk factor for LVH. CMA/B gene is not related with hypertensive LVH.

Keywords: Angiotensin-converting enzyme, chymases, essential hypertension, left ventricular hypertrophy, polymorphism

Introduction

Essential hypertension (EH) is one of the main diseases that cause the harm of human health. It is also the main risk factor related with cerebrovascular, coronary heart disease, heart failure and renal disease. As reported, EH is the leading risk factor related with the cause of death throughout the world, accounting for 12.8% of all deaths, including 51% of stroke deaths and 45% of coronary heart disease deaths [1]. The prevalence and mortality of EH increased year by year and EH has become the focus of the prevention and control of chronic non communicable diseases [2]. In recent decades, the prevalence of EH in China is also on the rise. EH is the most common chronic disease in China and also a major risk factor for cardiovascular disease, stroke, and kidney disease. The 2014 Chinese guideline for the management of hypertension is an update of the previous ones in 1999, 2005 and 2010. It showed that there was a 22% prevalence of hypertension in China, nearly 2 billion Chinese adults (aged ≥18 years), about one fifth of adults, had EH [3].

Left ventricular hypertrophy (LVH) is one of the complications of EH, and its presence has been associated with an increase in the incidence of heart failure, coronary artery disease, myocardial infarction, cardiac arrhythmias, and sudden death [4]. According to some statistics, LVH is considered a major predictor of cardiovascular morbidity and mortality. Growing studies showed that 20~40% of hypertensive patients have the complication of LVH, LVH is one of the most important characteristics of hypertension target organ damage and is also an independent cardiovascular risk factor in patients with EH [5, 6].
Cardiac hypertrophy is a compensatory response to cardiac insult of any cause [7]. It is the phenotypic consequence of interactions between genetic and non-genetic factors that involve multiple etiologies and complex mechanisms. Genes encoding proteins involved in the structure of the left ventricle and genes encoding cell signal transduction, hormones, growth factors, calcium homeostasis, and blood pressure are likely candidates for the development of LVH [8]. However, identification of specific genetic and molecular mechanisms for LVH in hypertensive patients is challenging [9, 10].

Renin-angiotensin-aldosterone system (RAAS) is the most important endocrine blood pressure control mechanism in our body, genes encoding components of this system have been strong candidates for the investigation of the genetic basis of hypertension. Angiotensin II (Ang II) is the final physiologically active product of RAAS. There are two major pathways producing Ang II in human tissues, angiotensin-converting enzyme (ACE) and chymase. They are involved in structural remodeling of the cardiovascular system. CMA is probably responsible for ACE-independent Ang II formation in human heart [11]. Ang II may indeed directly stimulate myocardial growth independent of the mechanical stress caused by its blood pressure-raising effect and lead to LVH in EH patients. So ACE and chymase, the two important pathways influenced the generation of Ang II, may play an important role in the LVH of EH patients. This study aimed to investigate the gene polymorphism of ACE2350 G/A and chymase gene CMA/B in EH patients and to explore the molecular genetic mechanisms of hypertensive LVH. On the basis of this observation, we hoped to provide clinical and experimental data for the prevention and control of LVH in patients with EH.

Materials and methods

Study populations

From Aug. 2010 to Jun. 2014, a total of 761 patients with EH were enrolled in the study. The EH patients were divided into LVH (EH with LVH) group and EH (EH without LVH) group basing on the fact of LVH or not. EH was diagnosed if a systolic blood pressure ≥140 mmHg and/or receiving antihypertensive treatment according to the 2014 Chinese guideline for the management of hypertension. Accordingly, a group of 382 gender, age, smoking history-matched healthy volunteers without history of hypertension served as controls (Control group). This study was approved by the Medical Ethics Committee of Taizhou People’s Hospital, and written informed consent was obtained from all participants.

Evaluation of LVH

Transthoracic echocardiography examinations were performed in all subjects in the partial left decubitus position with a S2.5-3.5 MHz transducer attached to a Doppler echocardiography machine (Phillips, IE-33). Evaluation of LVH was based on the criteria set by the American Society of Echocardiography. The following parameters was obtained as an average of at least 3 measurements: 1) left atrial diameter (LAD), 2) interventricular septum thickness (IVST), 3) left ventricular diastolic posterior wall thickness (LVPWT), 4) left ventricular diameter.
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Figure 1. The results of agarose gel electrophoresis (ACE for A, Chymase for B) and gene sequencing (ace2350 G/A for C, ACE2350G/G for D, ACE2350A/A for E, CMA/B G/A for F, CMA/B AA for G and CMA/B G/G for H).
Genetic polymorphisms with left ventricular hypertrophy

**Table 3.** Distributions of ACE 2350 G/A genotypes and alleles in LVH, EH and Control group

<table>
<thead>
<tr>
<th>Genotypes/Alleles</th>
<th>LVH group (n = 405)</th>
<th>EH group (n = 356)</th>
<th>Control group (n = 382)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>93 (22.9%)</td>
<td>107 (30.1%)</td>
<td>133 (34.8%)</td>
</tr>
<tr>
<td>GA</td>
<td>208 (51.4%)</td>
<td>171 (48.0%)</td>
<td>52 (13.4%)</td>
</tr>
<tr>
<td>AA</td>
<td>104 (25.7%)</td>
<td>78 (21.9%)</td>
<td>131 (21.9%)</td>
</tr>
<tr>
<td>G</td>
<td>197 (48.6%)</td>
<td>192 (53.9%)</td>
<td>230 (60.2%)</td>
</tr>
<tr>
<td>A</td>
<td>208 (51.4%)</td>
<td>164 (46.1%)</td>
<td>151 (39.5%)</td>
</tr>
</tbody>
</table>

**Table 4.** ACE 2350 G/A genotypes/alleles frequencies comparison in three groups

<table>
<thead>
<tr>
<th>Genotypes/Alleles</th>
<th>LVH-EH</th>
<th>LVH-Control</th>
<th>EH-Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>0.246</td>
<td>0.061</td>
<td>0.481</td>
</tr>
<tr>
<td>GA</td>
<td>0.624</td>
<td>0.994</td>
<td>0.621</td>
</tr>
<tr>
<td>AA</td>
<td>0.553</td>
<td>0.033*</td>
<td>0.132</td>
</tr>
<tr>
<td>G/A</td>
<td>0.272</td>
<td>0.016*</td>
<td>0.195</td>
</tr>
</tbody>
</table>

Compared with control group, *p<0.05.

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end diastolic diameter (LVEDD), 5) Left ventricular ejection fraction (LVEF). Physical examination such as height (H, cm) and body weight (W, kg) was recorded and body surface area (BSA) calculated as BSA (m²) = 0.0061×H+0.0128×W-0.1529. Left ventricular mass (LVM) was determined by the Devereux formula: LVM(g)=[1.04×[(IVST+LVPWT+LVEDD)³-LVEDD³]-13.6, while left ventricular mass index (LVMI) calculated as LVMI = LVM/BSA. LVM was defined as LVPWT >1.2 cm or if the LVMI ≥110 g/m² in women and 125 g/m² in men.

**SNP identification and genotype analysis**

Blood samples were collected after overnight fasting. Genomic DNA was extracted using standard protocols with Axygen Genomic DNA purification kit and stored at -80°C. The 2350 G/A genotype in the ACE and the CMA/B polymorphism in Chymase gene was determined by polymerase chain reaction (PCR) with the following program: 95°C for 30 s, followed by 35 cycles consisting of 30 s at 95°C, 30 s at 60°C and 30 s at 72°C, ended by a final extension at 72°C for 5 min. PCR products were then subjected to direct sequencing by GeneScript Co. LTD. Primers used for ACE 2350 G/A and CMA/B polymorphism detection and sequencing were listed in Table 1.

**Statistical analysis**

The data are expressed as means ± standard deviation of the mean. Un-paired student’s t-test was used to compare continuous variables, and chi-square test and Fisher’s exact test were used to compare categorical variables. Significant difference in variables between groups was determined with the Fisher exact test for categorical variables and the t test for continuous variables. Line correlation analysis was performed in correlation analysis, stepwise multiple regression and logistic regression model were used in performing multivariate analysis. All statistical tests were 2-sided with a 0.05 level of significance. Statistical analyses were performed using SPSS software (version 18.0, SPSS, Inc., Chicago).

**Results**

**Clinical characteristics**

The clinical characteristics of EH participants were listed in Table 2. There were no significant differences in gender, age, smoking history, diabetes history, SBP or DBP between LVH group and EH group.

**Genotype analysis**

The results of agarose gel electrophoresis and gene sequencing were shown in Figure 1. As shown in Tables 3 and 4, the distribution of ACE 2350 G/A genotypes (GG, GA, AA) was 22.9%, 51.4% and 25.7% for LVH group, 30.1%, 48.0% and 21.9% for EH group, and 34.8%, 51.4% and 13.9% for control group, respectively. The derived allele frequencies for the G and A allele were 48.6% and 51.4% in LVH group, 53.9% and 46.1% in EH group, and 60.2% and 39.5% in control group, respectively. Significant difference was only observed in AA genotype and A allele between LVH group and control group. Compared with control group, *p<0.05.

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and A allele were 77.1% and 22.9% in LVH group, 71.1% and 28.9% in EH group, and 69.98% and 30.1% in control group, respectively. No significant difference of genotypes distribution or alleles frequencies in any two groups, implying that CMA/B genotypes were not significantly associated with EH.

**Discussion**

EH is a common disorder affecting 20%-30% of the adult population in Western countries, whereas 27.2% of the adult Chinese population aged 35-74 years suffers from this disease [3, 12]. According to 2013 European Society of Hypertension/Cardiology guidelines, the presence of subclinical organ damage is a fundamental and important factor in determining the estimated cardiovascular risk with proposed scale [13]. LVH is one of common subclinical organ damage induced by hypertension, according to some statistics, 20-40% of hypertensive patients have the complication of LVH, the presence of LVH increases mortality of cardiovascular disease [5, 6]. Identifying specific risk factor index for hypertensive patients has therefore great potential for preventing adverse cardiovascular events.

A large body of evidence shows that the individual genetic background predisposes the LVH incidence [14]. ACE gene is one of the most intensely studied genes because of the key role it plays in the RAAS. The insertion deletion (I/D) polymorphism in this gene refers to an Alu repetitive sequence 287 bp long, in intron 16, resulting in three genotypes, DD and II homozygotes and ID heterozygotes. The I/D polymorphism is reported to determine circulating and tissue ACE levels and has recently been implicated in the pathogenesis of LVH in EH [15]. An exonic polymorphism 2350 G/A (rs4343) in angiotensin converting enzyme was shown to exert the most significant influence on plasma ACE levels. The ACE 2350 G/A polymorphism was in strong linkage disequilibrium and were independently associated with LVH [16, 17]. However, controversial results have been reported that the ACE 2350A allele is associated with a significantly reduced hypertension risk among Muslims from the Arab Gulf and Pakistan, yet an elevated risk among Han Chinese [18]. In this study, ACE2350 G and A allele frequency in LVH group were significantly higher than those in control group. There was a significantly correlation between ACE2350 gene polymorphisms and LVH in EH patients. AA genotype increases the risk of the occurrence of LVH. Our results are consistent with some reports describing that the polymorphism of ACE gene is associated with LVH in EH patients and A allele gene is a dangerous gene for LVH.

Chymase is one of the enzymes that produces angiotensin II (Ang II) and is a chymotrypsin-like serine protease that is abundant in the secretory granules of mast cells. Chymase gene CMA/B polymorphism (G/A transition at position -1903 of the 5’ untranscribed region of the gene) was found to have the effect on phenotypic expression of hypertrophic cardiomyopathy, but there were different results about the relationship between Chymase gene CMA/B polymorphism and LVH. As previously shown by Matsumoto et al, Chymase might play an important role in intermittent hypoxia-induced left ventricular remodeling, which is independent of the systemic blood pressure [19]. Li et al reported that the increase in Ang II levels via the dual pathway of Ang II formation by Chymase plays
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an important role in the cardiac hypertrophy of hamsters caused by the overloaded state and Chymase could be activated by mechanical stress in advance of an increase in its mRNA, and the Ang II level increased significantly [20]. The results of Gumprecht et al concerning the ACE I/D and Chymase gene CMA/B polymorphisms indicate that these polymorphic markers may at least in part represent a genetic predisposition to increased risk of the development of left ventricular hypertrophy in type 2 diabetic patients, with a possible interaction between these two loci [21]. However, controversial results have been reported that no evidence was found to support an association between Chymase gene CMA/B genotype and regression of LVH in patients or to support the interaction between ACE and Chymase gene in regression of LVH [22, 23]. In our study, there were no significant differences in both genotype and allele distribution of CMA/B polymorphism between EH group and LVH group, which indicated that CMA/B polymorphism was not associated with LVH in Chinese patients with EH. In summary, the results of our study concerning the ACE2350 G/A and Chymase CMA/B polymorphisms indicate that ACE2350 gene polymorphism was significantly correlated with LVH in Chinese EH patients, AA genotype increased the risk of the occurrence of LVH, A allele is a dangerous gene for LVH. There were no significant differences in both genotype and allele distribution of CMA/B polymorphism between EH group and LVH group. Since there were controversial results, and these controversial results may be involved the race region, sex, environment and the sample size, further more studies should help to confirm the presence of such relationship, and clarify its underlying molecular genetic mechanisms of LVH in patients with EH.

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Disclosure of conflict of interest

None.

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