Analysis of correlations between coronary heart disease and haplotypes of the angiotensin II receptor type 1 (AGTR1) gene

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ABSTRACT. This study aimed to explore correlations between haplotypes of the angiotensin II receptor type 1 (AGTR1) gene and coronary heart disease (CHD). In total, 204 patients with CHD and 206 healthy controls were genotyped using denaturing high-performance liquid chromatography between 2008 and 2014. Five polymorphic loci were found, namely, A-43281G, A-32954G, G-32839A, G-11064A, and A1880G. Likelihood estimates were used to identify haplotypes consisting of the A1166C locus and four of these five loci, then correlations between these haplotypes and CHD were assessed. Eight haplotypes with a frequency greater than 3% in the study population were discerned: ACCAA [odds ratio (OR) = 1.2381, 95% confidence interval (CI) = 0.7726-1.9843]; ACCCA (OR = 1.2604, 95%CI = 0.6104-2.6027); ACTAA (OR = 0.8929, 95%CI = 0.6607-1.2067); ACTAG (OR = 0.9274, 95%CI = 0.5692-1.5110); ATTAA (OR = 1.0347, 95%CI = 0.7505-1.4265); ATTAG (OR = 0.9110, 95%CI = 0.4227-1.9631); GCCAA (OR = 1.1273, 95%CI = 0.7259-1.7506); and GCTAA (OR = 0.7981, 95%CI = 0.4379-1.4546). However, the frequency of these
haplotypes did not significantly differ between CHD and the control groups. Thus, no correlation was established between the occurrence of CHD and AGTR1 haplotypes present at frequencies greater than 3%.

**Key words:** AGTR1; Gene polymorphism; Coronary atherosclerotic heart disease

**INTRODUCTION**

Coronary heart disease (CHD) is a cardiovascular disease (CVD) that represents a severe threat to public health. In the UK, 28% of all female deaths and 32% of all male deaths were due to CVD in 2012 (Bhatnagar et al., 2015). CHD has been shown to be a complicated disease involving multiple genes and is induced by environmental and hereditary factors. For instance, it has been found that polymorphisms such as PPARG rs1152002, angiotensin II receptor type 1 (AGTR1) rs5186, CXCL16 rs3744700, and LGALS2 rs7291467 may be closely related to its development (Tian et al., 2015). In addition, APLNR rs9943582, although not associated with coronary artery disease (CAD), is significantly associated with left ventricular systolic dysfunction among patients with this condition (Wang et al., 2015). In sudden cardiac death, which is primarily caused by CAD, genetic changes have also been implicated (Vatta and Spoonamore, 2015). Therefore, comprehensive understanding of CHD pathogenesis has become increasingly important for the diagnosis of this disease. With the rapid development of single nucleotide polymorphism (SNP) and genome-wide association studies, knowledge regarding the genetics of CHD has grown, and many candidate genes have been identified (Caproş et al., 2013). The renin-angiotensin system (RAS) is an important circulatory endocrine network that affects cardiovascular remodeling and maintains water-electrolyte homeostasis (Lis, 1999; Kumar et al., 2008). Polymorphism of RAS genes can affect the pathogenesis of hypertension and CAD, including ischemic heart disease and heart failure (Carluccio et al., 2001; Dhanachandra Singh et al., 2014). Furthermore, angiotensin is a critical component of the RAS and exerts its effects through AGTR1, which is related to vascular lesions. It is generally accepted that angiotensin II is involved in the pathogenesis of hypertension; less well recognized is its involvement in that of atherosclerosis (Rosei, 2008). Some studies have reported a correlation between AGTR1 genetic variants and CHD (Tiret et al., 1994; Abd El-Aziz et al., 2012; Li et al., 2013). In particular, several have demonstrated a close association between the A1166C polymorphism and CHD incidence (Nakauchi et al., 1996; Buraczynska et al., 2003; Abd El-Aziz et al., 2012). However, conflicting conclusions have also been reached (Alvarez et al., 1998). The present study principally consisted of screening for new AGTR1 SNPs, and investigating correlations between CHD and the haplotypes formed by these novel variant loci and A1166C.

**MATERIAL AND METHODS**

**Subjects**

According to the CHD diagnosis criteria of the World Health Organization, including disease history, symptoms, electrocardiography, and myocardial zymogram examination, 204 patients with coronary atherosclerotic heart disease having attended our hospital between 2008 and 2014 were enrolled in this study. Diagnoses for some of these patients were confirmed by coronarography. Of the patients enrolled, 146 had acute coronary syndrome, 22 suffered stable
angina pectoris, while 36 demonstrated remote myocardial infarction combined with chronic heart failure. A random sampling method was used to avoid inclusion of subjects with blood relationships. The control group consisted of 206 healthy volunteers without CHD chosen following physical examinations at our hospital. This study was conducted with approval from the Ethics Committee of Beijing Tongren Hospital of Capital Medical University, and all patients signed informed consent forms before participating.

Specimen collection and preparation

Fasting blood samples (2.7 mL) were collected, and 0.3 mL sodium citrate was added as an anticoagulant. Subsequently, DNA was extracted by alkaline lysis and dissolved in TE8 solution (tris-ethylenediaminetetraacetic acid, pH 8), before being stored at -20°C until needed. Total cholesterol (Chol), triglyceride, and high- and low-density lipoprotein cholesterol (HDL-C and LDL-C, respectively) levels were measured using a MODULAR P800 automatic biochemistry analyzer (Shanghai Roche Pharmaceutical Co., Ltd., Shanghai, China), and body mass index (BMI) was calculated (BMI = weight / height²).

Detection and selection of polymorphic loci

AGTR1 mRNA sequences were chosen from the National Center for Biotechnology Information/GenBank database (http://www.ncbi.nlm.nih.gov/nuccore/AF245699.1), in principal covering the regulatory region and five introns and exons. Denaturing high-performance liquid chromatography (dHPLC; Gallo et al., 2002; WAVE® system HPLC, Transgenomic, Inc., Omaha, NE, USA) was used to confirm mutations through blind screening. Twelve specimens from the CHD group and 12 from the control group were tested initially in order to identify mutant profiles for further sequence detection, using which, base changes at polymorphic loci were confirmed, and candidate loci with allele frequencies greater than 5% were chosen.

dHPLC was conducted as follows (Table 1 shows the primers and temperatures used). Polymerase chain reaction (PCR) products were placed onto the 96-well reaction plate of the WAVE system, before being sampled, detected, and analyzed. The volume of each sample was 3 µL. The buffer consisted of solution A [0.1M triethylammonium acetate (TEAA)] and B (25% acetonitrile in 0.1M TEAA). Table 1 shows the melting temperatures of the five polymorphic loci identified. Five samples with different dHPLC profiles were sequenced to confirm mutations.

<table>
<thead>
<tr>
<th>Table 1. Primers and temperature conditions used for denaturing high-performance liquid chromatography analysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphism</td>
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<tr>
<td>-----------------------</td>
</tr>
<tr>
<td>A-43281G</td>
</tr>
<tr>
<td>A-32954G</td>
</tr>
<tr>
<td>G-11064A</td>
</tr>
<tr>
<td>A-1196C</td>
</tr>
<tr>
<td>A1890G</td>
</tr>
</tbody>
</table>

TaqMan Universal kits (P/N43044437; Applied Biosystems, Waltham, MA, USA) were used
for gene sequencing, following the manufacturer protocol. PCR products were amplified using a GeneAmpPCR System 9700 thermal cycler and sequenced with an ABI PRISM7700 Sequence Detection System (both Applied Biosystems). The SDS software version 1.7 (Applied Biosystems) was used to analyze gene sequences and confirm three genotypes for every locus.

Six polymorphic loci were confirmed, including the polymorphisms A-43281G, A-32954G, G-32839A (exon 2), G-11064A (intron 2), A1166C (exon 5), and A1880G (at the 3’ end). However, A-32954G and G-32839A were found to be in complete linkage, therefore, of these two, only A-32954G was analyzed further.

**Statistical analysis**

Statistical analysis was performed using the SPSS17.0 software (SPSS Inc., Chicago, IL, USA) and data are reported as means ± standard deviations. The SHEsis software (Shi and He, 2005) was used to estimate haplotypes and conduct linkage disequilibrium analysis. Haplotypes consisting of five loci were analyzed and haplotype frequencies were calculated, including for those made up of four, three, and two loci. Haplotypes with frequencies greater than 3% were compared and D’ and r² values were calculated using the SHEsis software. Gender, genotype, and haplotype frequency distributions were analyzed by the chi-square test, while multi-factor analysis of variance was used to compare age, BMI, and biochemical indexes between groups. P < 0.05 was considered to denote a statistically significant difference.

**RESULTS**

**Biochemical measurements**

BMI, Chol, and LDL-C levels were significantly higher in the CHD group, whereas HDL-C measurements were markedly lower (P < 0.05; Table 2).

<table>
<thead>
<tr>
<th>Index</th>
<th>CHD (N = 204)</th>
<th>Control (N = 206)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>110/94</td>
<td>111/95</td>
<td>0.994</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.38 ± 12.41</td>
<td>61.26 ± 10.97</td>
<td>0.331</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.69 ± 2.49</td>
<td>25.89 ± 2.52</td>
<td>0.001</td>
</tr>
<tr>
<td>Chol (mM)</td>
<td>4.47 ± 1.11</td>
<td>4.22 ± 1.24</td>
<td>0.034</td>
</tr>
<tr>
<td>TG (mM)</td>
<td>1.76 ± 0.81</td>
<td>1.61 ± 0.93</td>
<td>0.077</td>
</tr>
<tr>
<td>LDL-C (mM)</td>
<td>3.44 ± 0.90</td>
<td>3.23 ± 0.95</td>
<td>0.019</td>
</tr>
<tr>
<td>HDL-C (mM)</td>
<td>1.16 ± 0.39</td>
<td>1.24 ± 0.42</td>
<td>0.049</td>
</tr>
</tbody>
</table>

CHD = coronary heart disease, BMI = body mass index, Chol = total cholesterol, TG = triglycerides, LDL-C = low-density lipoprotein cholesterol, HDL-C = high-density lipoprotein cholesterol.

**Comparisons of genotype frequencies**

Genotype frequencies of the five polymorphisms, including those of A1166C, were found to be in Hardy-Weinberg equilibrium, indicating that all subjects derived from the same population. The C allele of A1166C was clearly present at a higher frequency in the CHD group compared to the control group. However, alleles of the other polymorphisms were not found to significantly differ between the two groups (Table 3).
Coronary heart disease and AGTR1 haplotypes

Comparisons of haplotypes

Haplotypes present in more than 3% of the study population did not significantly differ in frequency between the two groups (Table 4).

Table 4. Comparisons of haplotypes.

<table>
<thead>
<tr>
<th>Haplotype (SNP combination)</th>
<th>CHD [N (%)]</th>
<th>Control [N (%)]</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCTAA</td>
<td>0.0420</td>
<td>0.0412</td>
<td>0.4386</td>
<td>1.1273 (0.7259-1.7602)</td>
</tr>
<tr>
<td>ACCCTAA</td>
<td>0.1199</td>
<td>0.1045</td>
<td>0.3901</td>
<td>1.1419 (0.7299-1.7709)</td>
</tr>
<tr>
<td>ACTATAA</td>
<td>0.2438</td>
<td>0.2428</td>
<td>0.9999</td>
<td>1.0347 (0.7505-1.4265)</td>
</tr>
<tr>
<td>ACTAGA</td>
<td>0.0312</td>
<td>0.0349</td>
<td>0.6188</td>
<td>0.9110 (0.4227-1.9631)</td>
</tr>
<tr>
<td>ATTAGA</td>
<td>0.0495</td>
<td>0.0563</td>
<td>0.5763</td>
<td>0.7581 (0.4378-1.3546)</td>
</tr>
</tbody>
</table>

CHD = coronary heart disease, OR = odds ratio, CI = confidence interval.

DISCUSSION

CHD is caused by environmental and hereditary factors, and constitutes a type of polygenic inheritance disease (Jia et al., 2012). The important RAS component angiotensin II is related to hypertension, a CHD risk factor. This protein is not only a vasoactive substance that affects blood pressure by regulating hemodynamics, but also a growth factor that plays a key role in CHD development, which is characterized by progressive fibrotic lesions of the vascular endothelium (Kim and Iwao, 2000). These biological effects are mediated by specific angiotensin II receptors, including AGTR1.

Previous studies have reported correlations between CVD and AGTR1 gene polymorphisms (Berdeli et al., 2005; Katsuya and Morishita, 2013), especially concerning the A1166C variant (Xu et al., 2010). This study principally focused on the discovery of new polymorphic AGTR1 loci, and investigated correlations between haplotypes of these and CHD. In this study, haplotypes were established using many adjacent SNPs and screened with the haplotype tag SNP method, an effective technique with a genome-wide range enabling the identification of genes involved in complicated diseases (Wall and Pritchard, 2003). This study described five new polymorphisms, and examined the association between their alleles and genotypes and CHD. Consistent with previous studies, we found that haplotypes consisting of four of these five novel SNPs and the A1166C variant exhibited no correlation with CHD. Thus, although a connection between the A1166C polymorphism and CHD was evident, the associated haplotypes showed no such relationship. Clearly, larger sample sizes should be included in further studies.
In summary, haplotypes comprised of the new AGTR1 polymorphisms described here and the A1166C sequence variant were not found to be correlated with CHD.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES


