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

L.J. Duan[1] and X.D. Wang[2]

[1]Department of Emergency, Beijing Tongren Hospital, Capital Medical University, Beijing, China
[2]Comprehensive Medical Department, Beijing Tongren Hospital, Capital Medical University, Beijing, China

Corresponding author: X.D. Wang
E-mail: xudongwangcn@126.com

Genet. Mol. Res. 15 (1): gmr.15017457
Received August 17, 2015
Accepted November 27, 2015
Published March 18, 2016
DOI http://dx.doi.org/10.4238/gmr.15017457

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; Buraczyńska 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 / height^2).

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 1. Primers and temperature conditions used for denaturing high-performance liquid chromatography analysis.

Polymorphism	Primer (5'→3')	Melting temperature (°C)
A-43281G	GTTGAAGAACACGAATCTCC	61
	GTTGCTGCTTCTTGGGTTC	
A-32954G	TGGCCATGTGGAGTCCTTG	56
	ACTGATGCCATCCCAGAAAG	
G-11064A	ACACTGTGGTGTAAATGGCTA	56
	CGCAAGTAGCCTAACATAGA	
A1166C	GAGAACATTCCTCTGCAGC	57
	CTCCTGTTGCTCCTCTAAC	
A1880G	ACTACTTGTAAAGGTGCTGC	53
	TCATACTCATTCAAGGTAGTC	

TaqMan Universal kits (P/N4304437; 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 r^2 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 2. Comparisons of biochemical measurements and demographic data for each study group (means ± standard deviations).

Index	CHD (N = 204)	Control (N = 206)	P value
Gender (male/female)	110/94	111/95	0.994
Age (years)	62.38 ± 12.41	61.26 ± 10.97	0.331
BMI (kg/m^2)	26.69 ± 2.49	25.89 ± 2.52	0.001
Chol (mM)	4.47 ± 1.11	4.22 ± 1.24	0.034
TG (mM)	1.76 ± 0.81	1.61 ± 0.93	0.077
LDL-C (mM)	3.44 ± 0.90	3.23 ± 0.95	0.019
HDL-C (mM)	1.16 ± 0.39	1.24 ± 0.42	0.049

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).

Table 3. Comparison of A1166C genotype and allele frequencies between the study groups (means ± standard deviations).

A1166C genotype or allele	CHD [N (%)]	Control [N (%)]	P value
AC	28 (13.7)	15 (7.3)	
CC	0 (0.0)	0 (0.0)	
AA	176 (86.3)	191 (92.7)	0.104
C	6.9%	3.7%	0.039

CHD = coronary heart disease. This table does not show comparisons of the genotype and allele frequencies of the other polymorphisms investigated in this study.

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.

	CHD	Control	P value	OR (95%CI)
ACCAA	0.1020	0.0859	0.3741	1.2381 (0.7726-1.9843)
ACCCA	0.0412	0.0337	0.5308	1.2604 (0.6104-2.6027)
ACTAA	0.2867	0.3166	0.4608	0.8929 (0.6607-1.2067)
ACTAG	0.0827	0.0905	0.7622	0.9274 (0.5692-1.5110)
ATTAA	0.2438	0.2428	0.8351	1.0347 (0.7505-1.4265)
ATTAG	0.0312	0.0349	0.8118	0.9110 (0.4227-1.9631)
GCCAA	0.1139	0.1047	0.5935	1.1273 (0.7259-1.7506)
GCTAA	0.0495	0.0625	0.4608	0.7981 (0.4379-1.4546)

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

Abd El-Aziz TA, Hussein YM, Mohamed RH and Shalaby SM (2012). Renin-angiotensin system genes polymorphism in Egyptians with premature coronary artery disease. *Gene* 498: 270-275. http://dx.doi.org/10.1016/j.gene.2012.02.033

Alvarez R, Reguero JR, Batalla A, Iglesias-Cubero G, et al. (1998). Angiotensin-converting enzyme and angiotensin II receptor 1 polymorphisms: association with early coronary disease. *Cardiovasc. Res.* 40: 375-379. http://dx.doi.org/10.1016/S0008-6363(98)00179-5

Berdeli A, Sekuri C, Sirri Cam F, Ercan E, et al. (2005). Association between the eNOS (Glu298Asp) and the RAS genes polymorphisms and premature coronary artery disease in a Turkish population. *Clin. Chim. Acta* 351: 87-94. http://dx.doi.org/10.1016/j.cccn.2004.08.015

Bhatnagar P, Wickramasinghe K, Williams J, Rayner M, et al. (2015). The epidemiology of cardiovascular disease in the UK 2014. *Heart* 101: 1182-1189. http://dx.doi.org/10.1136/heartjnl-2015-307516

Buraczyńska M, Pijanowski Z, Spasiewicz D, Nowicka T, et al. (2003). Renin-angiotensin system gene polymorphisms: assessment of the risk of coronary heart disease. *Kardiol. Pol.* 58: 1-9.

Caproş N, Barbacar N, Istrati V and Branişte T (2013). Aspects of the molecular-genetic profile in patients with ischemic heart disease. *Rev. Med. Chir. Soc. Med. Nat. Iasi* 117: 78-82.

Carluccio M, Soccio M and De Caterina R (2001). Aspects of gene polymorphisms in cardiovascular disease: the renin-angiotensin system. *Eur. J. Clin. Invest.* 31: 476-488. http://dx.doi.org/10.1046/j.1365-2362.2001.00839.x

Dhanachandra Singh Kh, Jajodia A, Kaur H, Kukreti R, et al. (2014). Gender specific association of RAS gene polymorphism with essential hypertension: a case-control study. *BioMed Res. Int.* 2014: 538053. http://dx.doi.org/10.1155/2014/538053

Gallo A, Thomson E, Brindle J, O'Connell MA, et al. (2002). Micro-processing events in mRNAs identified by DHPLC analysis. *Nucleic Acids Res.* 30: 3945-3953. http://dx.doi.org/10.1093/nar/gkf516

Jia EZ, Xu ZX, Guo CY, Li L, et al. (2012). Renin-angiotensin-aldosterone system gene polymorphisms and coronary artery disease: detection of gene-gene and gene-environment interactions. *Cell. Physiol. Biochem.* 29: 443-452. http://dx.doi.org/10.1159/000338498

Katsuya T and Morishita R (2013). Gene polymorphism of angiotensin II type 1 and type 2 receptors. *Curr. Pharm. Des.* 19: 2996-3001. http://dx.doi.org/10.2174/1381612811319170004

Kim S and Iwao H (2000). Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacol. Rev.* 52: 11-34.

Kumar R, Singh VP and Baker KM (2008). The intracellular renin-angiotensin system: implications in cardiovascular remodeling. *Curr. Opin. Nephrol. Hypertens.* 17: 168-173. http://dx.doi.org/10.1097/MNH.0b013e3282f521a8

Li Y, Li X, Jia N, Guo S, et al. (2013). Meta-analysis of the association between angiotensin II receptor, type 1 gene A1166C polymorphism and coronary artery disease in Chinese populations. *J. Renin Angiotensin Aldosterone Syst.* 14: 82-90. http://dx.doi.org/10.1177/1470320312450599

Lis GJ (1999). [Renal renin-angiotensin system in homeostatic water-electrolyte regulation]. *Przegl. Lek.* 56: 164-168.

Nakauchi Y, Suehiro T, Yamamoto M, Yasuoka N, et al. (1996). Significance of angiotensin I-converting enzyme and angiotensin II type 1 receptor gene polymorphisms as risk factors for coronary heart disease. *Atherosclerosis* 125: 161-169. http://dx.doi.org/10.1016/0021-9150(96)05866-2

Rosei EA (2008). Reduction of cardiovascular risk through angiotensin II type 1 receptor antagonism: focus on olmesartan medoxomil. *High Blood Press. Cardiovasc. Prev.* 15: 231-243. http://dx.doi.org/10.2165/0151642-200815040-00003

Shi YY and He L (2005). SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res.* 15: 97-98. http://dx.doi.org/10.1038/sj.cr.7290272

Tian J, Hu S, Wang F, Yang X, et al. (2015). PPARG, AGTR1, CXCL16 and LGALS2 polymorphisms are correlated with the risk for coronary heart disease. *Int. J. Clin. Exp. Pathol.* 8: 3138-3143.

Tiret L, Bonnardeaux A, Poirier O, Ricard S, et al. (1994). Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction. *Lancet* 344: 910-913. http://dx.doi.org/10.1016/S0140-6736(94)92268-3

Vatta M and Spoonamore KG (2015). Use of genetic testing to identify sudden cardiac death syndromes. *Trends Cardiovasc. Med.* 25: 738-748. http://dx.doi.org/10.1016/j.tcm.2015.03.007

Wall JD and Pritchard JK (2003). Haplotype blocks and linkage disequilibrium in the human genome. *Nat. Rev. Genet.* 4: 587-597. http://dx.doi.org/10.1038/nrg1123

Wang P, Xu C, Wang C, Wu Y, et al. (2015). Association of SNP Rs9943582 in APLNR with left ventricle systolic dysfunction in patients with coronary artery disease in a Chinese Han gene ID population. *PLoS One* 10: e0125926. http://dx.doi.org/10.1371/journal.pone.0125926

Xu M, Sham P, Ye Z, Lindpaintner K, et al. (2010). A1166C genetic variation of the angiotensin II type I receptor gene and susceptibility to coronary heart disease: collaborative of 53 studies with 20,435 cases and 23,674 controls. *Atherosclerosis* 213: 191-199. http://dx.doi.org/10.1016/j.atherosclerosis.2010.07.046