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Renin-angiotensin system gene polymorphisms and premature coronary heart disease

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Abstract

Introduction

Experimental and clinical studies demonstrated that the renin-angiotensin system (RAS) affects the pathogenesis of atherosclerosis and prognosis of coronary heart disease (CHD). The aim of this study was to investigate the genotype distribution and the allele frequencies of three RAS genes polymorphisms and their effects on premature CHD in a Turkish population.

Materials and methods

One-hundred and fifteen Turkish patients with premature CHD and 128 controls were included into the study. Angiotensin-converting enzyme (ACE), angiotensin II type 1 (AT₁) receptor and angiotensinogen (AGT) gene polymorphisms were analysed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Results

The patients group showed an increased frequency of the ACE D allele compared with controls (65% *vs.* 35%, $p=0.0001$). There was a significant association between the DD genotype and premature CHD (ACE DD *vs.* ID and II; odds ratio [OR]=2.82 [CI 95% 1.33–2.91, $p=0.002$]). Also, we observed increased premature CHD risk associated with higher frequencies of the AGT MM genotype in patients when compared with controls (AGT MM *vs.* TT and MT, OR=1.92 [CI 95% 1.11–3.33, $p=0.018$]). We found a significant association between AT₁-receptor AA genotype and decreased risk of premature CHD (AT₁R AA *vs.* AC and CC, OR= 0.57 [CI 95% 0.34–0.95, $p=0.03$]).

Conclusions

We demonstrated that increased premature CHD risk is associated with higher frequencies of the ACE DD and AGT MM genotypes. These findings indicate a synergistic contribution of ACE DD and AGT MM polymorphisms to the development of premature CHD. Also, our results suggest that family history, smoking, diabetes, hypertension, obesity and ACE DD genotype were independent risk factors for premature CHD.

Introduction

Cardiovascular disease (CVD) is a major public health problem in many societies. According to the most important epidemiological investigation in the Turkish population, the Coronary Heart

Diseases Risk Factors in Turkish Adults (TEKHARF) study, cardiovascular disease is the leading cause of mortality in Turkey.¹ Several types of CVD have a genetic basis, or at least a genetic component. It has been estimated that about 50% of the variability of the major risk factors for coronary heart disease (CHD) is genetic. Environmental factors, such as diet, also influence CVD and may interact with genetic factors to modulate risk.

Over the past decade, much interest has developed in the role of the renin-angiotensin system (RAS) in CVD. The RAS is not only essential in controlling cardiovascular haemodynamics and homeostasis, but also plays an important role in the development of CHD. Three major components of the RAS are angiotensinogen (AGT) the precursor of angiotensin I (Ang I); angiotensin-converting enzyme (ACE), which converts Ang I to angiotensin II (Ang II), the crucial biologically active product of the RAS; and the Ang II type-1 (AT₁)-receptor, the cell surface receptor for Ang II.²

Experimental and clinical studies demonstrated that the RAS affects the pathogenesis of atherosclerosis and prognosis of coronary artery disease.^{3–6} Previous studies have shown that the DD genotype of the ACE gene is associated with the development of left ventricular hypertrophy, myocardial infarction (MI) and remodelling. The TT genotype of the AGT gene is an independent risk factor for CHD,⁷ and individuals with AT₁-receptor CC genotype would be at increased risk of MI.⁸

In this study, we planned to investigate the genotype distribution and the allele frequencies of ACE, AGT and AT₁-receptor gene polymorphisms and their effects on premature CHD in Turkish population.

Materials and methods

The ACE, AGT and the AT₁-receptor gene polymorphism was analysed in 115 unrelated Turkish patients with a diagnosis of premature CHD, who were admitted to the Cardiology Department of the three centres in the Aegean region of West Turkey. The control group consisted of 128 unrelated healthy subjects without a history of CHD. The study was approved by the Ethics Committee of the Celal Bayar University hospital, and all subjects provided written informed consent. The inclusion criteria for the patients were: 1) age at

the time of CHD diagnosis 55 years or less in men and 65 years or less in women; 2) stenosis of at least 50% in a major coronary artery, or one of their branches, as determined by angiography. The extent of disease was defined as the number of arteries with stenosis at least 50% as single or multiple vessels. The coronary angiography was performed by Judkin's method at the Catheterization Laboratories. Diagnosis of MI was ascertained from patients records using the WHO criteria⁹ based on symptoms, elevation in cardiac enzymes or electrocardiographic changes.

All patients provided information about coronary risk factors, such as diabetes mellitus, hypertension, hypercholesterolaemia and cigarette smoking. Triglycerides, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels were measured by conventional methods of clinical chemistry. Arterial hypertension was defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg on more than one occasion. Patients with a history of diabetes or basal glycaemia >120 mg/dl were defined as diabetic. Smoking habit from all subjects was defined as a daily intake of more than five cigarettes. Body mass index (BMI) was defined as increased when >25 kg/m². A familial history of CHD was determined by interviewing patients and controls.

Genetic analysis

Genomic DNA was extracted from 200 μ l of EDTA-anticoagulated peripheral blood leucocytes, using the QUIAmp Blood Kit (QUIAGEN, Ontario Canada, Cat. no:51106). Amplification of DNA for genotyping the ACE I/D polymorphism was carried out by polymerase chain reaction (PCR) in a final volume of 15 μ l containing 200 μ M dNTP mix, 1.5 mM MgCl₂, 1x Buffer, 1 unit of AmpliTaq polymerase (PE Applied Biosystems) and 10 pmol of each primer. The primers used to encompass the polymorphic region of the ACE were 5'-CTG-GAGACCACTCCCATCCTTTCT-3' and 5'-ATGTG-GCCATCACATTTCGTCAGAT-3'.¹⁰

DNA was amplified for 35 cycles, each cycle comprising denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 1 minute with final extension time of 7 minutes. The initial denaturation stage was carried out at 95°C for 5 minutes. The PCR products were separated on 2.5% agarose gel and identified by ethidium-bromide staining. Each DD genotype was confirmed through a second PCR with primers specific for the insertion sequence.¹¹

To analyse AGT M235T polymorphism we PCR-amplified genomic DNA with primers 5'-GAT-GCGCACAAGGTCCTG-3' and 5'-CAGGGTGCT-GTCCACACTGGCTCGC-3', respectively. PCR consisted of 50 mM KCl, 2.5 mM MgCl₂, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 100 μ M of dNTP and 0.5 U of Taq DNA polymerase in a total volume of 50 μ l. After an initial denaturation at 94°C, followed by 25 cycles of 1 minute at 94°C, 1 minute at 61°C and 1 minute 72°C. PCR products were digested with the restriction enzyme SfaNI

Table 1 The demographic characteristic and distribution of risk factors in patients and control subjects

	Patients (n=115)	Controls (n=128)
Age (years)	48.1 \pm 7.9	44.2 \pm 7.4
Male/female	89/26	100/28
BMI (kg/m ²)	26.3 \pm 2.5	24.6 \pm 2.6
Smoking habit (≥ 5 per day)	77 (67%)	47 (37%)
Family history of CHD	49 (43%)	13 (10%)
Hypertension	44 (38%)	21 (16%)
Diabetes	24 (21%)	3 (2%)
Total cholesterol (mg/dL)	202.3 \pm 34.6	187.9 \pm 27.3
HDL cholesterol (mg/dL)	41.7 \pm 5.3	43.4 \pm 4.6
LDL cholesterol (mg/dL)	131.3 \pm 27.1	121.3 \pm 24.4
Triglycerides (mg/dL)	179.5 \pm 76.5	152.2 \pm 53.6
Single vessel disease	48 (42%)	-
Multiple vessel disease	67 (58%)	-

BMI = body mass index; CHD = coronary heart disease; HDL = high-density lipoprotein; LDL = low-density lipoprotein

and electrophoresed on a 3% agarose gel.¹²

The AT₁-receptor A1166C polymorphism was analysed with primers 5'-GCAGCACTTCACTAG-CAATGAT-3' and 5'-TGTTCTTCGAGCAGCCGT-3' as previously described.¹³

Statistical analysis

Statistical analysis was carried out with SPSS program for Windows 98 version 10.0 (SPSS Inc., Chicago, IL, USA). Variables are presented as means \pm SD. A p value of 0.05 or less was considered as significant. Univariate analysis was performed by Chi-squared, odds ratios (OR) and Mann Whitney U test. The Hardy-Weinberg's equilibrium for genotype distribution was estimated by the chi-squared test. We also calculated the genotypic OR for premature CHD and their 95% confidence interval (CI) with two-tailed p values, using multiple logistic regression analysis adjusted for family history of CHD, diabetes, hypertension, total cholesterol, triglycerides, HDL-C, LDL-C, BMI and smoking habit. The Backward Wald method was used for logistic regression analysis.

Results

The study population consisted of 115 patients with premature CHD and 128 control subjects. The clinical characteristics of the patients and controls are summarised in Table 1. The patients (77%) and controls (78%) were predominantly men. The patient group had a higher prevalence of hypertension, diabetes, smoking and family history of premature CHD than the controls. The patients also had higher total cholesterol, LDL-C and triglycerides levels and lower HDL-C level ($p < 0.05$). According to our results, family history, hypertension, diabetes, smoking, obesity, high total cholesterol, LDL-C and triglycerides levels and low HDL-C conferred an increased risk of premature CHD (Table 2).

Table 2 Odds ratios of traditional cardiovascular risk factors in premature coronary heart disease (CHD)

	OR (CI 95%)	p
Gender	0.91 (0.50–1.67)	0.77
Hypertension	3.35 (1.82–6.14)	0.001
Diabetes	10.99 (3.21–37.61)	0.001
Smoking	3.47 (2.05–5.89)	0.001
BMI (kg/m ²)	3.19 (1.88–5.42)	0.001
Family history of premature CHD	6.57 (3.32–12.99)	0.001
Total cholesterol	3.88 (2.03–7.42)	0.001
LDL cholesterol	1.99 (1.06–3.74)	0.030
HDL cholesterol	2.98 (1.56–5.67)	0.0001
Triglycerides	2.41 (1.25–4.64)	0.008

BMI = body mass index; HDL = high-density lipoprotein; LDL = low-density lipoprotein; OR = odds ratio

Table 3 Genotype distribution according to the presence of premature coronary heart disease (CHD)

	Patients (n=115)	Controls (n=128)	
ACE genotypes			
DD	52 (45.2%)	34 (26.4%)	$\chi^2=9.835$ p=0.007
ID	46 (40.0%)	63 (48.8%)	
II	17 (14.8%)	31 (24.0%)	
Allele frequencies I/D	0.348/0.652	0.492/0.508	-
AT1R genotypes			
AA	55 (47.8%)	79 (61.7%)	$\chi^2=9.089$ p=0.011
AC	50 (43.5%)	43 (33.6%)	
CC	10 (8.7%)	6 (4.7%)	
Allele frequencies A/C	0.695/0.305	0.785/0.215	
AGT genotypes			
MM	46 (40.0%)	33 (25.8%)	$\chi^2=5.145$ p=0.076
MT	42 (36.5%)	71 (55.5%)	
TT	27 (23.5%)	24 (18.8%)	
Allele frequencies M/T	0.583/0.417	0.535/0.465	

ACE = angiotensin-converting enzyme; AGT = angiotensinogen; AT1R = AT₁-receptor

The distribution of the ACE I/D, AGT T/M and AT₁-receptor A/C genotypes and allele frequencies in patients and controls were compatible with Hardy-Weinberg's equilibrium, as presented in Table 3 (ACE I/D: p=0.007, $\chi^2=9.835$; AT₁-receptor A/C p=0.011, $\chi^2=9.089$; AGT T/M: p=0.076, $\chi^2=5.145$). The patient group showed a significantly increased frequency of the ACE D allele compared with controls (0.652 *vs.* 0.508 p=0.0001).

There was a significant association between the DD genotype and premature CHD [ACE DD *vs.* ID and II; OR=2.82 (CI 95% 1.33–2.91, p=0.002)]. Also, we observed increased premature CHD risk associated with higher frequencies of the AGT-MM genotype in patients when compared with controls [AGT MM *vs.* TT and MT, OR=1.92 (CI 95% 1.11–3.33, p=0.018)] and a significant association between AT₁-receptor AA genotype and decreased risk of premature CHD [AT₁-receptor AA *vs.* AC

Table 4 Odds ratios of ACE, AT₁-receptor and AGT genotypes among premature coronary heart disease (CHD)

Genotypes interaction	OR (CI 95%)	p
ACE II versus ID and DD	0.53 (0.28–1.05)	0.065
ACE DD versus II and ID	2.82 (1.33–2.91)	0.002
AT1R AA versus AC and CC	0.57 (0.34–0.95)	0.03
AT1R CC versus AA and AC	1.94 (0.68–5.51)	0.21
AGT-MM versus TT and MT	1.92 (1.11–3.33)	0.018
AGT-TT versus TM and MM	1.33 (0.72–2.47)	0.37
ACE-DD and AT1R-CC	8.23 (1.00–67.95)	0.021
ACE-DD and AT1R-AC	2.95 (1.35–6.51)	0.006
ACE-ID and AT1R-AA	0.51 (0.27–0.94)	0.031
ACE-ID and AGT-TM	0.43 (0.22–0.81)	0.008
ACE-II and AGT-MM	0.28 (0.77–1.05)	0.46
ACE-DD and AGT-MM	3.75 (1.60–8.77)	0.001
ACE-DD and AGT-TT and AT1R-AA	0.46 (0.12–1.83)	0.26

ACE = angiotensin-converting enzyme; AGT = angiotensinogen; AT1R = AT₁-receptor; OR = odds ratio

Table 5 Adjusted* odds ratios for cardiovascular risk factors in premature coronary heart disease (CHD)

Risk factors	OR (95% CI)	p
Family history of premature CHD	6.92 (3.04–15.79)	0.001
Hypertension	2.52 (1.13–5.60)	0.023
Diabetes mellitus	10.84 (2.70–43.55)	0.001
Smoking	4.58 (2.31–9.08)	0.008
BMI (kg/m ²)	2.88 (1.49–5.57)	0.002
ACE-DD	2.74 (1.39–5.42)	0.004
AT1R-AA	0.59 (0.31–1.14)	0.12
AGT-MM	1.95 (0.95–3.97)	0.067

* Logistic Regression; ACE = angiotensin-converting enzyme; AGT = angiotensinogen; AT1R = AT₁-receptor; BMI = body mass index

and CC, OR=0.57 (CI 95% 0.34–0.95, p=0.03)] (Table 4).

By multiple regression analysis, family history of premature CHD, smoking, diabetes, hypertension, obesity and ACE DD genotype were independent risk factors for CHD. The patients with ACE DD genotype had a 2.7-fold higher risk of CHD when compared with the control group [OR=2.74 (CI 95% 1.39–5.42, p=0.004)] (Table 5).

The effect of the ACE DD genotype on the risk of premature CHD was higher in AT₁-receptor C allele carriers. The odds ratios for premature CHD associated with the ACE DD genotype were considered as 2.95 (CI 95% 1.35–6.51, p=0.006) in AT₁-receptor AC heterozygotes and 8.23 (CI 95% 1.00–67.95, p=0.021) in AT₁-receptor CC homozygotes. The effect of ACE DD genotype on the risk of premature CHD was higher in AGT MM genotype subjects (ACE DD and AGT MM: OR=3.75 [CI 95% 1.60–8.77, p=0.001]) (Table 4).

The ACE, AT₁-receptor and AGT genotypes were not associated with the number of affected

