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The influence of angiotensin-converting enzyme 2 gene polymorphisms on type 2 diabetes mellitus and coronary heart disease

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Abstract. – BACKGROUND: Diabetes mellitus is a chronic metabolic disorder that results from various genetic and environmental factors, and type 2 diabetes mellitus (T2DM) combined with coronary heart disease (CHD) is one of the most common chronic complications in diabetes.

AIM: To explore the correlation between the polymorphism of angiotensin-converting enzyme 2 (ACE2) and T2DM combined with CHD.

PATIENTS AND METHODS: A total of 120 patients with T2DM and 93 patients with T2DM and CHD were selected to participate in this study. And polymerase chain reaction -restriction fragment length polymorphism (PCR-RFLP) was used to analyze the polymorphism of G8790A in ACE2 gene. Meanwhile, the clinical, biochemical and echocardiographic data were also analyzed.

RESULTS: There was no significant difference between T2DM group and T2DM combined with CHD group in genotype and allele frequencies ($p > 0.05$). And for different genotypes, both groups had no significant difference in the age, BMI, blood lipid, fasting blood glucose, EF ratio, E/A ration, LVPWTd and LVEDd ($p > 0.05$). But for male patients in T2DM combined with CHD group, the IVSTd, LVPWTd and urine protein level of allele G were significantly higher than that of allele A with p values less than 0.01, 0.05 and 0.05, respectively.

CONCLUSIONS: Genetic polymorphism of G8790A in ACE2 gene plays an important role on the pathogenesis of male patients with T2DM and CHD by changing the levels of IVSTd, LVPWTd and urine protein. Therefore, our results reveal the role of ACE2 polymorphism in the pathogenesis of T2DM with CHD and identify several potential biomarkers for this disease

Key Words:
Angiotensin-converting enzyme 2, Polymorphism, Type 2 diabetes mellitus, Coronary heart disease.

Introduction

Type 2 diabetes mellitus (T2DM) is believed to be caused by many genetic and environmental factors as well as their interactions. Genetic genes possibly involve in many steps of its pathogenesis, in particular the excessive activation of the renin-angiotensin system (RAS). RAS is activated on the early stage of the T2DM. As the main products of the activated RAS, ANGII has been proved to be important in the pathogenesis of T2DM[1]. Coronary artery disease (CAD) in patients with type 2 diabetes mellitus (T2DM), which is one of the common chronic complications in diabetic patients, seriously affects the patients' life and quality of life. And the pathogenic mechanism is currently unknown. The interdiction of RAS is propitious to avoid or delay the occurrence of the T2DM and to reduce the occurrence of cardiovascular and renal disease[2]. Angiotensin-converting enzyme 2 (ACE2) is discovered in 2000, which is the new member of the RAS family. ACE2 metabolize the ANGII[3] by competing with angiotensin converting enzyme (ACE) and ACE2 gene has been found to be polymorphic in human populations. It is known that there are 140 SNP loci in human ACE2 gene. To investigate the affection and association of ACE2 gene polymorphisms to the pathogenic mechanism of the T2DM+CHD, we detect the polymophisms of ACE2-G8790A gene on the T2DM+CHD in the Han Chinese population in Guangdong region.

Patients and Methods

Patients

We performed a case-control study to randomly collect the blood of the patients with T2DM from Endocrinology Department, Clinic and Cardiovascular Internal Medicine. These blood samples were diagnosed and genotyped according to the World

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Health Organization/Western Pacific Region (WHO/WPR) standard in 1999. The patients were all examined with Color Doppler Ultrasound and Routine 12 Leads ECG or exercise ECG and ambulatory electrocardiogram without hepatic-renal disease caused by hypertension and non-diabetes. Partial cases were examined with coronary angiography or 64-slice spiral computed tomography (CT) coronary angiography. All the patients needed to assay HbA1c, urinary albumin, blood lipid, renal function and so on and to record blood pressure and body mass index (BMI). 213 cases were collected with clinical symptoms and myocardial ischemia after auxiliary examination or poor cardiac systolic and diastolic functions. The patients, who had ever clinical diagnosed coronary heart disease or myocardial infarction, were defined as group CHD. The CHD group contained 93 cases (the male 44 cases and female 49cases). The non-CHD group as control contained 120 cases (the male 51 cases and female 69 cases).

Genetic Analysis

Genomic DNA was extracted from sodium citrate peripheral bloods using the DNA extraction kit (TIANGEN Corporation, Beijing, China). DNA concentration was detected by ultraviolet spectrophotometer, and then we standardized DNA concentration to 20 ng/μl.

The forward primer 5'-CATGTGGTCAAAAGGATATGT-3' and the reverse primer 5'-AAAGTAAGGTTGGCAGACAT-3' to ACE2-G8790A gene (Genbank no.AY217547) were designed by Oligo 6.0. PCR was performed on grads PCR instrument (Biometra Corporation, Goettingen, Germany) in a total reaction volume of 25 μl consisting of 1μl genomic DNA template, 2.5 μl 10×PCR buffer, 25 μmol/L $MgCl_2$ 1.5 μl, 10 mmol/L dNTP 0.5 μl, 0.8 μl forward primer, 0.2 μl Taq DNA polymerase and 18.5 μl double distilled water. PCR program consisted of initial denaturation for 2 min at 95°C followed by 34 cycles of denaturation for 30 sec at 94°C annealing at 50.6°C for 30 sec and synthesis at 72°C for 45 sec, and a final extension time for 7 min. The PCR products were detected by agarose electrophoresis directly. It confirmed the polymorphism sites of ACE2-G8790A, which was also the AluI restriction site (AGCT), located in the fourth base of intron 3 after the fragments sequencing (Figure 1). The volume of endonuclease reaction was 20 μl containing 2U AluI, 10×buffer 2 μl, PCR product 5 μl. After digestion for 16 hours at 37°C, finished by 6×Loading Dye and identified its genotype on 2% agarose electrophoresis.

Statistical Analysis

Statistical analysis was performed by the SPSS 11.5 package (SPSS Inc., Chicago, IL, USA). The data were expressed as mean±standard deviation. The genotype frequency was calculated respectively from cases of male and female in every group using ^{2} test. Mean comparison in different group were using homogeneity of variance and one-way ANOVA. Mean comparison in group was using independent *t*-test. $p < 0.05$ was considered statistical significant.

Results

The Decision of ACE2-G8790A Gene Polymorphism

The PCR product of ACE2 was 466bp, and different genotypes appeared different fragments after digesting by AluI. 174bp and 72bp fragments appeared in AA genotype; 466bp, 281bp and 185bp fragments appeared in AG genotype; only 466bp fragments appeared in GG genotype (Figure 2).

Genotype and Allele Frequencies of CAD+T2DM Group and T2DM Group

Genotype frequencies were the different frequencies of AA genotype, AG genotype and GG

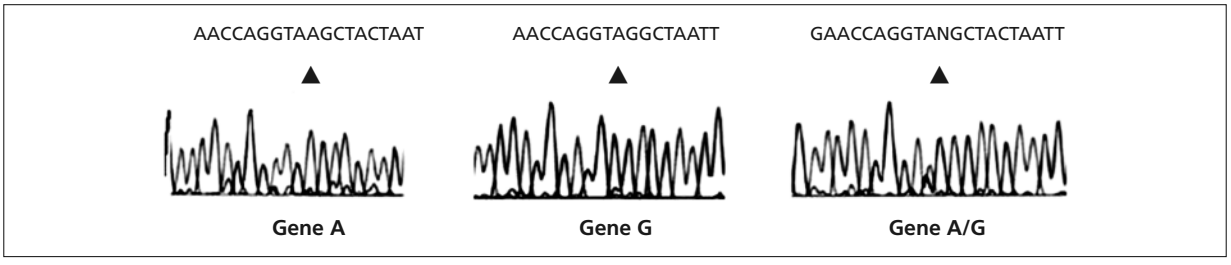

Figure 1. SNP of ACE2-G8790A sequencing results. ▲Polymorphic loci; single peak represented homozygote; double peak represented heterozygote.

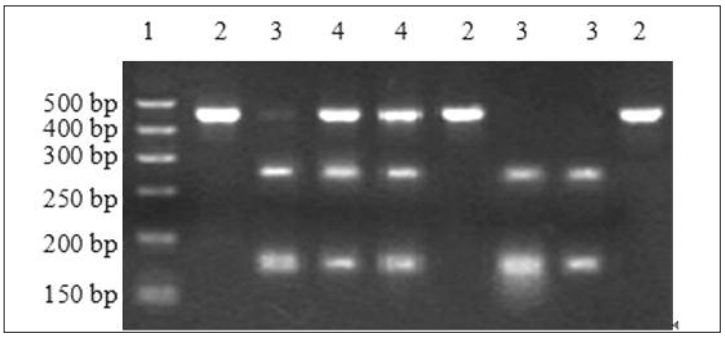

Figure 2. Restriction digests of ACE2-G8790A. ▲1: Marker; 2: GG genotype; 3: AA genotype; 4: AG genotype.

genotype. Allele frequencies were the different frequencies between allele A and allele G. The difference of genotype and allele frequencies were all no statistical significance using χ^2 test ($p > 0.05$) (Table I).

The Association of the clinical, Biochemical and Echocardiographic Data and ACE2 Genotype

The association of ACE2 gene polymorphism and echocardiographic parameters in male and female patients were shown separately in Table II and III respectively. The age, BMI, blood lipids, fasting blood glucose and $HbAI_C$ were all no statistical significance ($p > 0.05$) between T2DM+CHD group and T2DM group for different genotype. The ejection fraction (EF), (arly/late (atrial) ratio, E/A, LVPWTd, LVEDd were also no statistical significance ($p > 0.05$). But the IVSTd and LVPWTd of allele G were higher than allele A in male patients in T2DM+CHD group ($p < 0.01$, $p < 0.05$). Meanwhile allele G was higher than allele A on urine protein level. These data was partial normal distribution, and it had statistical significance comparing with allele A after taking logarithm as 10 was base ($p > 0.05$).

Discussion

There has been extensive interest in the association of ACE2 gene polymorphism and cardiovascular system disease in recent years. This gene with 18 exons locate in gene Xp22[4]. ACE2 gene polymorphism associated with left ventricular hypertrophy of early cardiac failure male patients' survival rate[5,6], reported in literature. 159 SNP loci of ACE2 gene in human and the majority of them locate in noncoding region of introns to date, especially G8790A gene polymorphism have been studied more, which locate in intron 3 abut upon exon. Frojdo et al[7] observed there was no association between single nucleotide polymorphisms (SNP) of ACE2-G8790A and diabetic nephropathy in Type 1 Diabetes Mellitus patients in Finland. Benjafield et al[8] studied in the association of 4 SNP loci contained G8790A and essential hypertension, and there was no positive association. But the results in the Han Chinese population were different. Recently, it reported ACE2-G8790A was associated with blood pressure elevation in metabolic syndrome patients[9]. Wei Yang's[10] study indicated ACE2 might act as a protective protein for cardiovascular diseases and common genetic variants in the ACE2 gene might impact on MI in females. It might possibly interact with alcohol consumption to affect the risk of CHD and MI in Chinese males. So there was racial difference in the association of ACE2 gene polymorphism and disease. And ACE2- G8790A gene polymorphism may associate with cardiac insufficiency in the Han Chinese population.

Table I. Genotype frequencies of CAD in patients with T2DM group and T2DM group [n (%)].

	Allele frequencies of male		Genotype frequencies of female		
Group	A	G	AA	AG	GG
T2DM	24 (47.06)	27 (52.94)	27 (39.13)	19 (27.54)	23 (33.33)
T2DM+CHD	19 (43.18)	25 (56.82)	13 (22.4)	20 (44.9)	16 (32.7)
χ^2	0.716		0.27		
p	0.416		0.876		

Table II. The association of ACE2 gene polymorphism and echocardiographic parameters in female patients.

	T2DM Group			T2DM+CDH Group		
	AA	AG	GG	AA	AG	GG
Age	49.80 ± 2.78	53.64 ± 3.83	53.58 ± 1.63	56.54 ± 1.98	55.90 ± 11.21	53.88 ± 1.70
Diabetic duration (year)	7.00 ± 6.04	7.94 ± 5.51	9.01 ± 5.43	8.85 ± 5.04	9.11 ± 5.39	8.38 ± 4.15
BMI (kg/m^2)	22.91 ± 2.69	23.06 ± 3.60	24.37 ± 3.42	23.31 ± 2.93	23.07 ± 3.49	24.18 ± 2.97
Fasting blood glucose (mmol/L)	8.53 ± 3.88	7.89 ± 3.83	7.22 ± 2.45	9.68 ± 2.89	8.64 ± 2.58	8.61 ± 2.42
HbAlc	7.49 ± 2.12	7.51 ± 2.77	6.96 ± 2.47	8.85 ± 2.23	8.52 ± 2.34	8.30 ± 2.13
Urine microprotein	81.71 ± 201.17	44.34 ± 102.78	49.37 ± 70.24	106.69 ± 183.67	88.55 ± 54.33	53.81 ± 106.24
TG (mmol/L)	2.05 ± 1.00	2.32 ± 1.26	2.03 ± 1.00	2.15 ± 0.70	2.33 ± 1.10	2.41 ± 1.07
TC (mmol/L)	5.26 ± 1.40	5.32 ± 1.47	5.19 ± 1.52	5.45 ± 1.79	5.32 ± 2.22	4.94 ± 1.66
HDL-C (mmol/L)	1.12 ± 0.38	1.10 ± 0.34	1.05 ± 0.35	1.02 ± 0.49	0.99 ± 0.45	1.01 ± 0.67
LDL-C (mmol/L)	3.00 ± 1.53	2.95 ± 1.44	2.77 ± 1.38	2.98 ± 1.45	2.71 ± 1.38	3.35 ± 1.67
EF (%)	54.37 ± 9.63	52.84 ± 9.04	51.35 ± 7.664	50.62 ± 7.80	51.25 ± 7.15	53.88 ± 7.68
E/A ratio (%)	80.09 ± 18.23	84.86 ± 19.01	81.66 ± 18.45	71.15 ± 20.651	81.10 ± 18.43	80.06 ± 17.98
IVSTd (mm)	0.91 ± 0.13	0.93 ± 0.18	0.95 ± 0.18	1.00 ± 0.21	1.00 ± 0.13	0.98 ± 0.17
LVPWTd	0.94 ± 0.12	0.92 ± 0.21	0.92 ± 0.16	0.99 ± 0.15	1.00 ± 0.14	0.97 ± 0.14
LVMI (g/m^2)	79.96 ± 9.18	77.84 ± 11.76	78.61 ± 11.97	81.85 ± 12.24	81.55 ± 12.45	78.19 ± 13.20
LVEDd (mm)	4.71 ± 0.61	4.86 ± 0.66	4.73 ± 0.67	4.88 ± 0.74	4.79 ± 0.71	4.99 ± 0.65

BMI: body mass index; HbAlc: glycosylated hemoglobin; TC: total glyceride; TG: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; BUN: blood urea nitrogen; IVSTs: nterventricular septal end- systolic thickness; IVSTd: nterventricular septal end-diastolic thickness; PWTs: left ventricular posterior wall end- systolic thickness; PWTd: left ventricular posterior wall end-diastolic thickness; LVMI: the left ventricular mass; LVEDd: left ventricular end-diastolic diameter.

ACE2 gene is on the X-chromosome. We also estimate the association of ACE2- G8790A gene polymorphism and clinical characteristics, echocardiographic parameters in T2DM+CDH patients. Age, diabetic duration, BMI, fasting blood glucose, blood lipid, HbAlc are all no statistical significance in T2DM group of male and female patients. E/A ratio, Ejection fraction, LV-

Table III. The association of allele ACE2 and echocardiographic parameters in male patients.

	T2DM group		T2DM+CDH group	
	A	G	A	G
Age	52.31 ± 11.99	53.49 ± 12.48	53.31 ± 10.65	54.54 ± 11.53
Diabetic duration (year)	8.42 ± 4.89	8.42 ± 4.89	8.50 ± 4.56	8.67 ± 5.15
BMI (kg/m^2)	23.31 ± 3.69	23.31 ± 3.69	23.80 ± 2.80	24.18 ± 2.72
Fasting blood glucose (mmol/L)	7.29 ± 2.42	7.29 ± 2.42	7.91 ± 3.17	7.97 ± 2.74
HbAlc	6.83 ± 2.51	6.83 ± 2.51	8.34 ± 2.23	8.85 ± 2.95
Urine microproteins	62.80 ± 106.68	62.80 ± 106.68	67.03 ± 122.80	143.87 ± 255.75*
TG (mmol/L)	2.07 ± 1.00	2.07 ± 1.00	2.57 ± 0.88	2.65 ± 0.79
TC (mmol/L)	5.25 ± 1.51	5.25 ± 1.51	5.28 ± 1.47	5.41 ± 2.05
HDL-C (mmol/L)	1.12 ± 0.38	1.12 ± 0.38	1.21 ± 0.53	1.17 ± 0.40
LDL-C (mmol/L)	2.78 ± 1.35	2.78 ± 1.35	2.94 ± 1.57	3.27 ± 1.48
EF (%)	53.67 ± 9.63	53.67 ± 9.63	52.55 ± 7.18	51.08 ± 7.50
E/A ratio (%)	82.54 ± 19.69	82.54 ± 19.69	81.00 ± 18.47	80.42 ± 19.23
IVSTd (mm)	1.09 ± 0.17	1.09 ± 0.17	1.11 ± 0.24	1.31 ± 0.21#
LVPWTd	1.04 ± 0.19	1.04 ± 0.19	1.05 ± 0.17	1.10 ± 0.23
LVMI (g/m^2	80.50 ± 11.72	80.50 ± 11.72	82.40 ± 11.73	89.93 ± 11.37

#Compared with AA genotype in group, $p < 0.01$; *Compared with AA genotype in group, $p < 0.05$.

MI, IVST, thickness of left ventricular free wall, LVEDd are also no significant difference. We get the same conclusion in female patients in T2DM+CHD group. Among the male patients in T2DM+CHD group, IVSTd is significantly greater in patients of GG genotypes than AA genotypes ($p < 0.01$), and LVMI are also significantly higher in GG genotypes ($p < 0.05$). But EF and E/A ratio are no significant difference, so it shows the ACE2-G8790A polymorphism associate with early cardiac hypertrophy. The pathogenic mechanism of the T2DM+CHD affected by the ACE2 gene polymorphism is not clear recently. AngII is the key product in RAS, which can promote vasoconstriction, accelerate cell growth-hypertrophy, participate in cell apoptosis, improve endothelial cells and vascular smooth muscle cells hyperplasia[11]. AngII plays the role of gluconeogenesis and glycogenolysis. Blood glucose elevations stimulate insulin secretion and induced insulin resistance[12,13]. As new member of RAS, ACE2 has high expression on heart, blood vassela, kidneys and testis. It is important for adjustment of blood pressure and maintenance of heart function, which is also associated with myocardial infarction, heart failure and diabetes mellitus[14]. It converts AngI and AngII into Ang1-9 and Ang1-7, but the production of Ang1-7 directly hydrolyzed from AngII is 400 times of Ang1-9 approach. Thus, the main function of ACE2 is metabolizing AngII and antagonism of negative effect in T2DM and CHD from AngII.

80% of T2DM patients die of cardiovascular diseases and 3/4 of them die of coronary disease. The diabetes patients have high risk of coronary heart disease[15]. The incidence of T2DM+CHD is 1-2 and 3-4 times of non-diabetes population in male and female patients respectively and it is early onset, severe clinical symptoms. The mortality of heart diseases in diabetes patients is 10-20 times to non-diabetes under 45 years old and the advantages in female disappear. The incidence of CHD is significantly lower in female (before menopause) than male in non-diabetes population, and there are no gender differences in diabetes patients. Schuster et al[16] found ACE gene was only associated with Acute Myocardial Infarction (AMI) in female. Lindpaintner et al[17] found ACE gene was no associated with AMI among the all-male subject and its pathogenic mechanism was not clear. Our data show IVSTd, LVNI and urine protein is increasing in allele ACE2-G in male patients. As the homologous gene of ACE, ACE2 gene is on the X-chromosome, so ACE2 polymorphism may cause gender differences in incidence of T2DM+CHD. Urine protein is partial normal distribution, and allele ACE2-G is higher than allele ACE2-A after taking logarithm as 10 was base in male patients in T2DM+CHD group ($p < 0.05$). It points out ACE2 polymorphism associate with urine protein in T2DM+CHD group. These researches show micro-proteinuria is the independent cardiovascular risk factor and cause of death, also the low level urine protein increases the incidence of CHD and death[18]. Endothelial injury increases arterial wall permeability of lipid particle, so it lead to atherosclerosis and the leakage of albumin from kidney and all the vessels is also increasing. The differences of urine protein in different genotype male patients not only prompt early diabetic nephropathy, but also reflect the degree of vascular endothelial dysfunction.

Conclusions

We detect the ACE2 polymorphism and find gene mutation associated with IVSTd, LVMI and urine protein in male patients. But it couldn't distinguish if this locus changes the gene function or it is just a genetic marker. Many genes have atypia in RAS containing ACE, AT1R and so on. These genes combined ACE2 gene has different effect on biological effectors. The bias of selected subjects and the number of the sample are also influence the research conclusions. It need further study on the association of ACE2 gene combined other gene and T2DM+CHD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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