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Original Article

Association of *ACE* polymorphism and diabetic nephropathy susceptibility

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Abstract: Aim: The study was aimed to analyze whether *ACE* rs267604983 polymorphism was related to the onset of diabetic nephropathy (DN). Methods: 80 DN patients and 78 healthy controls were enrolled in the study. The differences of age, sex, body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP) between two groups were analyzed. The genotyping of *ACE* rs267604983 was conducted by the technology of PCR-HRM. Odds ratio (ORs) with 95% CI were used to evaluate the relationship of *ACE* rs267604983 with DN susceptibility. Results: The AA genotype of *ACE* rs267604983 was remarkably associated with the risk for DN (OR = 2.90, 95% CI = 1.12-7.51). In addition, for the A allele carriers, the risk for DN increased 1.87 fold (OR = 1.87, 95% CI = 1.16-3.01). The subgroup analysis showed that the AA genotype was found higher in normal albuminuria group than other groups (P = 0.006), while AG genotype was higher in macro albuminuria group (P = 0.036). Conclusion: *ACE* rs267604983 polymorphism is associated with the risk of DN. AA genotype and A allele may increase the risk for DN. Furthermore, AA and AG genotypes may have effects on the subgroups of DN.

Keywords: *ACE*, diabetic nephropathy, polymorphism, susceptibility

Introduction

Diabetic nephropathy (DN) is classified as a microvascular complication of diabetes mellitus (DM) with the diagnosis name of nodular diabetic glomerulosclerosis. With the prolonging of lifetime and the popularization of Western lifestyles, DN continues to get gradually worse and stage renal disease (ESRD) caused by DN has become the main threatening factor for DM patients. Not only is the onset of DN related with inappropriate changes of renal hemodynamics, activation of protein kinase C, activation of hexosamine biosynthesis pathway, activation of aldose reductase pathway and the formation of advanced glycation end products (AGE), but also it is related with abnormal changes of genes structure and function [1, 2]. It has been confirmed that the occurrence of DN exhibits the tendency of familial aggregation and hereditary factor plays an important role in DN pathogenesis. The etiology of DN has always been the hot spot of the research in this field, however, no clear etiology and pathogenesis have been found.

For the etiology, some studies have postulated that the onset of DN is associated with various genes and pathways [3-9]. And angiotensin converting enzyme (*ACE*) gene is one of important genes among them. *ACE* gene encodes ACE enzyme in the renin-angiotensin system (RAS), which plays a crucial role in blood pressure homeostasis [10-13]. Recent studies have suggested that *ACE* polymorphisms are also closely related with DN [14, 15]. However, there were few studies focusing on the association of *ACE* rs267604983 and DN. Therefore, we conducted a study to analyze the association of rs267604983 of *ACE* gene and DN susceptibility.

Materials and methods

Subjects

80 DN patients and 78 healthy checkers who were unrelated Chinese Han population were selected from our hospital at the same time. The subjects of control group ever suffered in diabetes, abnormality of glucose tolerance,

Table 1. Characteristics differences between case and control group

Variables	Case n = 80 (%)	Control n = 78 (%)	*P* value
Age	51.35 ± 1.51	51.74 ± 2.00	0.875
Male/female	32 (40.0)/48 (60.0)	29 (37.2)/49 (62.8)	0.716
BMI	26.29 ± 1.39	25.33 ± 1.14	0.597
SBP (kPa)	15.34 ± 0.80	14.65 ± 0.82	0.552
DBP (kPa)	10.44 ± 0.10	10.69 ± 0.15	0.160

hypertension and coronary heart disease were excluded from the study. DN patients were divided into three groups according to Urinary Alb/Cr Rate (UACR): normal albuminuria (UACR < 30 mg/g), micro albuminuria (30 mg/g ≤ UACR < 300 mg/g) and macro albuminuria (UACR ≥ 300 mg/g). The research program was approved by the Hospital Ethical Committee (HEC) of Shandong Provincial Hospital Affiliated to Shandong University and informed consent was obtained from the participants.

Genome DNA extraction

Five mL venous blood was collected from the participants with empty stomach in early morning. Genome DNA was extracted using DNA extraction kit (Beijing Tiangen biochemical technology co., LTD). The quantity and quality of DNA was checked with ultraviolet spectrophotometer and electrophoresis.

PCR-HRM

Based on the promoter sequences of *ACE* gene, the primers were designed by Primer Premier 5.0. Primer sequences were 5'-CTGGAGGAGGCAGGTAATGT-3' (forward) and 5'-CCACATTACCTGCCTCCTCC-3' (reverse). 20 μL PCR reaction system included 2 × SsoFast EvaGreen Supermix 10 μL (Bio-Rad), 10 μmol/L forward and reverse primer 1 μL, 50 ng genome DNA and deionized water. PCR amplification was performed under the following conditions: predenaturation at 98°C for 5 min, 40 cycles of denaturation at 98°C for 5 s, annealing at 60°C for 5 s and extension at 72°C for 10 s, followed by 98°C for 30 s and 72°C for 30 s with the incensement of 0.2°C forever 10 s from 70°C to 90°C. Then the corresponding curve chart of the melting-points was made. After the amplification, the standard curve was adjusted by Precision Melt software. PCR products were directly sequenced (Shanghai Personal Biotechnology co., LTD).

Statistical analysis

The representation of the subjects was tested using Hardy-Weinberg equilibrium (HWE). χ^2 test was used to compare the variables frequencies in two groups. The frequencies of genotypes and alleles were calculated by direct counting. Odds ratio (OR) with 95% confidence interval (95% CI) were calculated by χ^2 test. All the analysis were completed in SPSS 18.0. $P < 0.05$ indicated the statistical significance.

Results

Subject characteristics

Before the study, we analyzed the differences of age, sex, BMI, SBP and DBP between cases and controls (**Table 1**). The average age of case group was 51.35, while the control group was 51.74. The distributions of females accounting for 60.0% in the cases and 62.8% in the controls, were little higher than that of males (40.0% and 37.2%). There were no significant differences of BMI, SBP and DBP between two groups (P: 0.597, 0.552 and 0.160).

Genotypes and alleles distribution of ACE rs267604983

The results of PCR-HRM showed that *ACE* rs267604983 polymorphism contained three genotypes: AA, GG and AG. As shown in **Table 2**, the differences of genotypes and alleles frequencies of rs267604983 were remarkable between the case and control group. The striking differences of AA genotype between cases and controls suggested that AA genotype could increase the risk for DN (OR = 2.90, 95% CI = 1.12-7.51). In addition, we found that the A allele in case group was higher than that of control group, which indicated that for A allele carrier, the risk for DN increased 1.87 fold (OR = 1.87, 95% CI = 1.16-3.01). Then, we analyzed the association of genotypes distribution of rs267604983 between DN subgroups (**Table 3**). The results suggested that the AA genotype was found higher in normal albuminuria group than other groups ($P = 0.006$), while AG genotype was higher in macro albuminuria group ($P = 0.036$).

Table 2. Comparison of gene frequency in case and control group

Genotypes/alleles	Case (%)	Control (%)	X^2	P	OR (95% CI)
GG	33 (41.2)	45 (57.7)	--	--	1.00
AG	30 (37.5)	25 (32.0)	1.938	0.164	1.64 (0.82-3.28)
AA	17 (21.3)	8 (10.3)	5.003	0.025	2.90 (1.12-7.51)
G	96 (60.0)	115 (73.7)	--	--	1.00
A	64 (40.0)	41 (26.3)	6.699	0.010	1.87 (1.16-3.01)

Table 3. Genotypes distribution between the subgroups of DN

Genotypes	Case			Control	*P* value		
	Normal (%)	Micro (%)	Macro (%)		Normal	Micro	Macro
GG	4 (28.6)	15 (55.6)	14 (35.9)	45	--	--	--
AG	5 (35.7)	6 (22.2)	19 (48.7)	25	0.248	0.545	0.036
AA	5 (35.7)	6 (22.2)	6 (15.4)	8	0.006	0.182	0.149

Notes: Normal for normal albuminuria group, Micro for micro albuminuria group, Macro for macro albuminuria group.

Discussion

DN is one of the most important complications of DM patients, which includes a progressive increase in urinary albumin excretion and an increase in blood pressure [16]. For the mechanism of DN, the studies indicated that RAS is associated with the progression of DN [17-21].

ACE gene locates on the 23 area at long arm of 17 chromosome (17q23), including 26 exons and 25 introns. *ACE*, decoded by *ACE*, is a key enzyme in RAS, which can cause vasoconstriction by generating angiotensin II and inactivating bradykinin. After combining to the angiotensin II receptor, ACE can raise blood pressure through the excretion of aldosterone, increased content of sodium retention, the excretion of arginine vasopressin (AVP) and adrenocorticotropic hormone (ACTH) in pituitary and release norepinephrine of sympathetic nerve. Therefore, we assumed that *ACE* was significantly associated with the development of DN. Further studies have showed that *ACE* polymorphism is also related to the onset of DN [7, 22-24], however, there was no study focusing on the association of *ACE* rs267604983 and DN susceptibility. Therefore, we explored the relationship *ACE* rs267604983 with DN susceptibility based on the population of Jinan city, Shandong province.

In our study, we adopted the method of PCR-HRM which is a new technology for screening and analyzing mutation compared with the traditional PCR-RFLP technique. The technology can easily identify the wild type and mutant individuals. Until now, it has become one of the important methods for gene mutation detection [25]. Our studies suggested that AA genotype and A allele of rs267604983 were significantly associated with onset of DN. The subgroup analysis showed that the frequencies of AG and AA genotypes were respectively higher in macro albuminuria group and normal albuminuria group, which indicated that AG and AA genotypes might be the genetic-susceptibility factors of macro albuminuria and normal albuminuria, respectively.

In conclusion, *ACE* rs267604983 polymorphism could increase the risk for DN. As we all know, complicated genetic susceptibility to the diseases is influenced by multiple genes and environmental factors. Since the differences in the district and population ethnicity, much more well-designed study should be conducted to testify the conclusion.

Disclosure of conflict of interest

None.

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