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Original Article

Association between *RAGE* gene polymorphisms and the susceptibility of diabetic nephropathy

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Abstract: Objective: The objective of this study was to investigate the association of receptor for advanced glycation end products (*RAGE*) gene polymorphisms and the risk of diabetic nephropathy (DN). Methods: Genotypes of *RAGE* gene polymorphisms (rs1800624, rs1800625 and rs2070600) were genotyped by MassArray method. Hardy-Weinberg equilibrium (HWE) was utilized to detect the representative of the cases and controls. Genotype and allele frequencies were obtained by direct calculation. Differences of genotypes and alleles of the polymorphisms between the case and control groups were assessed by Chi-square test. SPSS 18.0 was used to carry out all of the calculations. Results: Genotype and allele frequencies of rs1800624, rs1800625 and rs2070600 were higher in type I DN group that in control group, but the differences were not significantly (P>0.05). In type II diabetic patients, rs1800624 and rs1800625 polymorphisms were not related to the occurrence of DN (P>0.05). A allele of rs2070600 polymorphism might act as a risk factor for the development of DN in type II diabetic patients (P=0.032, OR=1.520, 95% CI=1.036-2.229). In the total DN patients (both in type I and type II DM) no significant association was observed in rs1800624 polymorphism and the development of DN. But both C and A alleles of rs1800625 and rs2070600 were significantly related to the susceptibility of DN (P=0.030, OR=1.515, 95% CI=1.040-2.209; P=0.043, OR=1.405, 95% CI=1.010-1.956). Conclusion: Rs1800624 was not related to the development of DN. *RAGE* rs1800625 and rs2070600 polymorphisms were associated with the total DN risk.

Keywords: Diabetes mellitus, diabetic nephropathy, *RAGE*, polymorphisms

Introduction

Along with the upturn living standards, the changes on dietary patterns and life expectancy, the incidence of diabetes mellitus (DM) has an uptrend. Diabetic nephropathy (DN) is the most common complication of DM [1]. DN is characterized by kidney glomeruli and glomerulosclerosis. DN is a chronic progressive kidney disease which is caused by angiopathy of the kidney glomeruli. It is one of the leading cause of renal failure in patients who suffer from the end-stage renal disease (ESRD) [2, 3]. Recent years, DN has become one of the epidemic disease all around the world, threatening the human health, and resulting in large loss in economy. Unfortunately, there was no effective therapy methods to completely cure DN. In order to find out an effective treatment method of DN, we should investigate the etiology of it.

It is reported that the occurrence and development of DN is influenced by many factors [4-8]. Many studies demonstrated that blood glucose could affect the occurrence of DN [9, 10]. However, some diabetes patients who had a poor long term blood glucose control did not suffer from the diabetes complication. In contrast, someone who had a good blood glucose control might suffer from diabetes complication [10]. Obvious difference is found in the incidence of the DN among different ethnicity. Familial aggregation tendency also present in the occurrence and development of DN. These phenomena have shown that genetic factor play an crucial role in the occurrence of DN [11]. Recent years, a lot of researches were carried out in this area, and multiple susceptibility gene was found [12-15], including receptor for advanced glycation end products (*RAGE*) gene [16].

Table 1. Characteristics of participants

Features	CON n=127	Type I DN n=112	Type II DN n=108
Age (mean ± SD)	59.57±15.72	44.3±8.98	63.1±10.21
Gender			
Male	61	54	52
Female	65	58	56
Disease duration (years)	-	17.98±6.87	18.15±8.27

Protein encoded by *RAGE* gene, advanced glycation endproducts (AGEs) receptor, is a member of the immunoglobulin super family of cell surface receptors. AGE receptor is first characterized by Neeper et al. in 1992 [17]. After *RAGE* is found, the function of it is investigated. It was surmised that *RAGE* might relate to diabetic complication. But the association of *RAGE* gene with the susceptibility of DN is still unclear. So in this study we explored the role of *RAGE* gene polymorphisms (rs1800624, rs1800625 and rs2070600) in the occurrence of DN.

Materials and methods

Subjects

A total of 347 Chinese Han subjects comprising three groups were enrolled in this study: 1) healthy controls without any disease (CON); 2) type I diabetics with DN (I-DN); 3) type II diabetics with DN (II-DN). Diabetic patients were all diagnosed in diabetic outpatient in The Second Affiliated Hospital of Harbin Medical University. Both diabetics and non-diabetics were age- and gender-consistency among groups. Informed consent form was obtained from all participants. This study was in accordance with the Declaration of Helsinki and approved by the ethic committee of The Second Affiliated Hospital of Harbin Medical University. Features of participants are listed in **Table 1**.

Detection of polymorphisms

Genomic DNA was isolated from peripheral blood by a GenElute™ Blood Genomic DNA Kit (Sigma, USA). *RAGE* gene polymorphisms (rs1800624, rs1800625 and rs2070600) were detected using a MassARRAY Analyzer 4 system (Sequenom, USA) following the manufacture's instruction.

Statistical methods

Representativeness of the cases and controls was detected by Hardy-Weinberg equilibrium (HWE) examination. Genotype and allele frequencies of *RAGE* gene polymorphisms were obtained by direct calculation. Genotype and allele differences of the polymorphisms among the groups were assessed by Chi-square test. All of the calculations were performed by SPSS 18.0. Odds ratios (ORs) with corresponding 95% confidence intervals (CIs) were used to represent the relative risk of DN. $P<0.05$ was considered statistical significance.

Results

HWE test

RAGE gene polymorphisms (rs1800624, rs1800625 and rs2070600) were genotyped and shown in **Table 2**. Allele distributions in controls did not deviated from HWE examination, indicating the goodness of fit for the controls.

The role of RAGE gene polymorphisms (rs1800624, rs1800625 and rs2070600) independently in type I and type II DN

Genotype and allele frequencies of the three polymorphisms were shown in **Table 2**. We found that A, C and A alleles of rs1800624, rs1800625 and rs2070600 were higher in type I DN group than that in healthy controls. But genotype and allele distributions of the three *RAGE* gene polymorphisms had no obvious differences in type I DN patients and healthy controls ($P>0.05$). The same results were observed in rs1800624 and rs1800625 polymorphisms with the developing of DN in type II diabetic patients ($P>0.05$). No significant associations existed in GA and AA genotype of rs2070600 polymorphism with the occurrence of DN in rs2070600 polymorphism ($P>0.05$). But A allele of rs2070600 polymorphism was significantly associated with the developing DN in type II diabetic patients ($P=0.032$, OR=1.520, 95% CI=1.036-2.229).

Association of RAGE gene polymorphisms (rs1800624, rs1800625 and rs2070600) with the susceptibility of DN

We analyzed the association of *RAGE* gene polymorphisms with the total DN risk (**Table 3**), so as to get a more consistent result. The results showed that both genotype and allele of

Table 2. *RAGE* gene polymorphisms (rs1800624, rs1800625 and rs2070600) and DN independently in type I and type II DM

SNP	CON n=127	I-DN n=112	*P*	OR (95% CI)	II-DN n=108	*P*	OR (95% CI)
rs1800624							
TT	85 (66.93)	69 (61.61)	-	-	63 (58.33)	-	-
TA	39 (30.71)	42 (37.50)	0.304	1.327 (0.774-2.275)	41 (37.96)	0.209	1.418 (0.821-2.449)
AA	3 (2.36)	1 (0.89)	0.201	2.464 (0.594-10.212)	4 (3.70)	0.447	1.799 (0.389-8.324)
T	209 (82.28)	180 (80.36)	-	-	167 (77.31)	-	-
A	45 (17.72)	44 (19.64)	0.589	1.135 (0.716-1.800)	49 (22.69)	0.180	1.363 (0.866-2.143)
rs1800625							
TT	81 (63.78)	58 (51.79)	-	-	56 (51.85)	-	-
TC	43 (33.86)	49 (43.75)	0.085	1.591 (0.936-2.705)	46 (42.59)	0.111	1.547 (0.904-2.649)
CC	3 (2.36)	5 (4.46)	0.248	2.328 (0.535-10.129)	6 (5.56)	0.069	3.522 (0.841-14.751)
T	205 (80.71)	165 (73.66)	-	-	158 (73.15)	-	-
C	49 (19.29)	59 (26.34)	0.066	1.496 (0.972-2.301)	58 (26.85)	0.051	1.536 (0.996-2.369)
rs2070600							
GG	61 (48.03)	43 (38.39)	-	-	38 (35.19)	-	-
GA	56 (44.09)	58 (51.79)	0.159	1.469 (0.860-2.511)	55 (50.93)	0.104	1.577 (0.910-2.733)
AA	10 (7.87)	11 (9.82)	0.352	1.560 (0.609-3.999)	15 (13.89)	0.051	2.408 (0.982-5.904)
G	178 (70.08)	144 (64.29)	-	-	131 (60.65)	-	-
A	76 (29.92)	80 (35.71)	0.178	1.301 (0.887-1.909)	85 (39.35)	0.032	1.520 (1.036-2.229)

rs1800624 had no significant association with the susceptibility of DN ($P>0.05$). Similar results existed in the genotypes of rs1800625 and rs2070600 with the total DN risk ($P>0.05$). But significant associations were observed in C and A alleles of rs1800625 and rs2070600 with the susceptibility of DN ($P=0.030$, OR=1.515, 95% CI=1.040-2.209; $P=0.043$, OR=1.405, 95% CI=1.010-1.956).

Discussion

Normal AGEs level in healthy people will increase with the age. In diabetic patients, this reaction may be accelerated. AGEs could lead to multiple biological effects which will damage the organism [18-20]. *RAGE* is the receptor of AGEs [21]. Combination of AGEs-*RAGE* could lead to multiple effects including oxidative stress, cell dysfunction and the conformation of NF-κB, then play a potential role in the occurrence of type II DM and the complications of it [22, 23]. In the hyperglycemia state, AGEs level is enhanced. Simultaneously, in the AGEs accumulation parts, the expression level of *RAGE* has a rising trend [24]. Interaction between AGEs and *RAGE* is an important factor for diabetes-associated metabolic memory [25]. Recent study reported that *RAGE* gene presented an up-regulation in diabetic atherosclerosis [26]. It is believed that *RAGE* is a candidate gene for the investigation of various of vascular diseases.

Diabetes is one of the serious chronic disease, the main danger for this disease is caused by various complications. DN is one of microvascular complications for diabetes. Because diabetes could divided into two groups (type I and type II), the DN also occurrence from the two types of diabetes. Based on the above point, we speculated that DN may be influenced by *RAGE* gene. *RAGE* gene is located at 6p21.3, and contains 11 exons. A lot of polymorphisms were found in *RAGE* gene. Single nucleotide polymorphisms (SNPs) of *RAGE* gene relate to many diseases. Two SNPs in *RAGE* gene promoter region, -374T/A (rs1800624) and -429T/C (rs1800625), have been reported to affect the transcription activity [27]. A Gly to Ser alteration in the 82th codon in exon 3 (Gly82Ser, rs2070600) is associated with the occurrence of inflammation and vascular disease [28, 29]. Previous studies showed that *RAGE* gene polymorphisms might contribute to the occurrence and development of DN [30]. However, the pathogenesis of DN is still unknown. So in this study, we investigated the role *RAGE* gene play in the etiology of DN.

In this study, minor alleles frequencies of three *RAGE* gene SNPs (rs1800624, rs1800625 and

Table 3. Association of *RAGE* gene polymorphisms (rs1800624, rs1800625 and rs2070600) with the susceptibility of total DN

	CON n=127	Total-DN n=220	*P*	OR (95% CI)
rs1800624				
TT	85 (66.93)	132 (60.00)	-	-
TA	39 (30.71)	83 (37.73)	0.186	1.370 (0.858-2.188)
AA	3 (2.36)	5 (2.27)	0.924	1.073 (0.250-4.608)
T	209 (82.28)	347 (78.86)	-	-
A	45 (17.72)	93 (21.14)	0.277	1.245 (0.838-1.848)
rs1800625				
TT	81 (63.78)	114 (51.82)	-	-
TC	43 (33.86)	95 (43.18)	0.054	1.570 (0.992-2.485)
CC	3 (2.36)	11 (5.00)	0.138	2.605 (0.704-9.636)
T	205 (80.71)	323 (73.41)	-	-
C	49 (19.29)	117 (26.59)	0.030	1.515 (1.040-2.209)
rs2070600				
GG	61 (48.03)	81 (36.82)	-	-
GA	56 (44.09)	113 (51.36)	0.075	1.520 (0.958-2.411)
AA	10 (7.87)	26 (11.82)	0.097	1.958 (0.879-4.364)
G	178 (70.08)	275 (62.50)	-	-
A	76 (29.92)	165 (37.50)	0.043	1.405 (1.010-1.956)

rs2070600) were different between type I DN groups and control group, which indicated that the three polymorphisms had no obvious association with the development of DN in type I diabetes. This was partly according to previous studies performed by Poirier et al. [31], which analyzed the rs1800624 polymorohsm. But no researches were focused on the associations of rs1800625 and rs2070600 polymorphisms with the susceptibility of DN in type I diabetes. For the developing DN in type II diabetes, rs1800624 and rs1800625 polymorphisms did not relate to the susceptibility of DN. While, A allele of rs2070600 polymorphism was significantly associated with 1.520 times increased risk of DN in type II diabetic patients. That was different from previous studies [32, 33]. We also detected the association of total DN susceptibility with the *RAGE* gene polymorphisms. No apparent relationship was observed in *RAGE* rs1800624 polymorphism and the susceptibility of DN in total DM. That was according to the results respectively in type I and type II diabetes. Interestingly, C allele of rs1800624 SNP significantly increased the risk of DN with the OR of 1.515 that was completely different from the association in type I and type II diabetes respectively. Meanwhile, A allele of rs2070600 was a significantly risk factor for the occurrence of DN in total diabetes and type II DM patients, but not in type I diabetic patients.

As we all known, apparent differences of DN incidence existed in different ethnicity [34]. The most important finding for our study was that *RAGE* gene polymorphisms might increase the risk of DN in Chinese Han population. However, because the unadjusted data, the evidence obtained from this study was inadequate to certify the etiology of DN.

Disclosure of conflict of interest

None.

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