Original Article

Association of fibronectin Msp iv polymorphism and diabetic nephropathy susceptibility in Chinese Han population

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Abstract: Aim: Our study was aimed to study the distributional characteristics of fibronectin (Fn) Msp iv polymorphism in Chinese Han Population and investigate its association with susceptibility and clinicopathologic features of diabetic nephropathy (DN). Methods: Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were applied to testify Fn Msp iv genotypes among 108 patients with DN and 86 healthy individuals. Odds ratio (OR) with 95% confidence interval (CI) were used to evaluate the association of Fn Msp iv polymorphism and onset risk and clinicopathologic stages of DN. Results: The comparison of genotype and allele distribution in normal, micro and massive proteinuria groups showed that genotype and allele distribution in massive proteinuria group showed great differences, compared with those of control group (P = 0.006, P = 0.004). Further analysis on the association of Fn Msp iv polymorphism and occurrence of abnormal proteinuria suggested that DD genotype and D allele appeared to be a risk factor for abnormal proteinuria (OR = 3.553, 95% CI = 1.278-9.875; OR = 2.442, 95% CI = 1.378-4.327). Then, we analyzed the effects of Fn Msp iv polymorphism on the clinicopathologic stages of DN, the result showed that DD genotype showed great effect on the occurrence of early-onset DN (OR = 7.500, 95% CI = 1.691-33.272). For the DN patients with D allele, the risk for early-onset DN was increased 3.445 folds (OR = 4.445, 95% CI = 1.869-33.10.574). Conclusion: Fn Msp iv polymorphism appeared to be associated with DN susceptibility.

Keywords: Diabetic nephropathy, fibronectin, polymorphism, susceptibility

Introduction

Diabetic nephropathy (DN), one of common microvascular complications of diabetes [1], is a common cause of end stage renal disease (ESRD). The occurrence of DN accompanied high level of pressure, lipids, sugar, protein and viscosity in blood and the microcirculation disturbance cause arteriosclerosis of kidney glomerulus, then result in the occurrence of edema and proteinuria [2-4]. Nevertheless, its pathogenesis is still unclear [5, 6]. For the etiology, it has been confirmed that DN are commonly caused by gene-environmental interactions and there exists variances in disease susceptibility for different populations with certain genetic background. Until now, the relationship of genes and DN has attracted lots of attention [7-10].

Fibronectin (Fn), a kind of protein called insoluble cryoglobulin, was found in low temperature precipitation of human blood in 1948, which was separated and purified by Mosesson in 1970. Until now, some studies have demonstrated that Fn was a multifunctional protein [11-14]. However, the studies about Fn mostly focus on protein level and few studies have investigated the function of Fn gene in the pathogenesis of DN.

Our research studied the distributional characteristics of Fn Msp iv, analyzed the association of Fn Msp iv and clinicopathologic features and
lastly evaluated the relationship of Fn Msp iv polymorphism with DN in Chinese Han population.

**Materials and methods**

**Patients and samples**

We chose 108 patients with diabetes including 51 males and 57 females hospitalized in Department of Endocrinology of the affiliated hospital of Qingdao University from March, 2012 to June, 2014. The average age of the patients was 58.4±11.9. The patients were all Chinese Han population and required no consanguinity and family history of diabetes.

Patients with type II diabetes were divided into three groups according to the ratio of urine protein and creatinine. If the ratio of urinary albumin and creatinine was 0.10 to 0.20, the patients were divided into normal proteinuria group (n = 58), and if the ratio was 0.21 to 0.30, the patients were divided into microalbuminuria group (n = 40). In addition, if the ratio was more than 0.31, the patients were divided into massive proteinuria group (n = 10). Groups of microalbuminuria and massive proteinuria were collectively called abnormal proteinuria group.

DN patients are diagnosed with microalbuminuria and massive proteinuria. Meanwhile, DN patients were divided into late-onset or potential group (30 cases) and early-onset group (20 cases). 86 healthy individuals were enrolled including 41 males and 47 females. The average age of the controls was 53.6±8.1. And the unrelated controls were required without histories of diabetes, high blood pressure, coronary heart disease and family history of diabetes.

**Polymerase chain reaction (PCR)**

2 ml peripheral venous blood was extracted from each subject (anticoagulation with EDTA), then stored under -20°C for use. DNA was extracted with the method of salt fractionation and genotyping was performed by PCR-RFLFP technology.

Primers were designed by Primer 5.0 software and synthesized by Shanghai Sangon Biotech co., LTD. The primer sequences were as the followings: 5’-GCC TGG TAC AGA ATA TGT AGT G-3’ (Forward); 5’-TGC CAT TAA GAG CAA CGA TCG-3’ (Reverse). PCR reaction mixture included 1 μl template, 1 μl dNTP, 1 μl forward primer, 1 μl reverse primer, 1 μl TaqDNA polymerase (5 U/μl), 1.5 μl MgCl₂ (25 mmol/L), 2.5 μl 10× Buffer, 13.8 μl double-distilled water. PCR reaction was performed under the following conditions: predegeneration at 94°C for 7 min, 32 cycles of degeneration at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 1 min and finally extension at 72°C for 10 min. PCR products were testified using 2% of agarose gel, and the results were observed and recorded with Uvipro gel imaging system.

**Restriction fragment length polymorphism (RFLP)**

PCR products were mixed with restriction enzyme and buffer solution. Msp iv genotypes were divided into three, genotype with a stripe was wild type CC, with three stripes was heterozygote CD, and with two stripes was mutant type DD.

**Statistical analysis**

Differences in genotype distribution of Fn Msp iv in case and control group were tested by Fisher's exact test. Odds ratio (OR) with 95% confidence interval (95% CI), calculated by χ², were used to evaluate the association of Fn Msp iv genotypes and the risk of DN. The relationship of clinical characteristics with Fn Msp iv genotypes was analyzed by Pearson χ² test. P < 0.05 indicates significant difference. Statistical analysis was performed using SPSS 18.0.

**Results**

**Hardy-Weinberg equilibrium (HWE) test**

Genotype distributions of Fn Msp iv in control group were complied with HWE (P > 0.05).

Genotypes and alleles distributions in groups of normal proteinuria, microalbuminuria, massive proteinuria and control group

There existed no significant differences in genotype and allele distribution between control and normal proteinuria group (P = 0.088, P = 0.223). Compared with control group, genotype distribution in microalbuminuria group also showed no significant difference (P = 0.150, P = 0.179). In addition, we also found that genotype and allele distribution in massive proteinuria group showed great differences, compared
Genotype and allele distribution in groups of normal proteinuria, microalbuminuria, massive proteinuria and control group

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Genotype, n (%)</th>
<th>Allele, n (%)</th>
<th>( \chi^2 )</th>
<th>P</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC</td>
<td>CD</td>
<td>DD</td>
<td>C</td>
<td>D</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>40 (46.5)</td>
<td>36 (41.9)</td>
<td>10 (11.6)</td>
<td>116 (67.4)</td>
<td>56 (32.6)</td>
<td></td>
</tr>
<tr>
<td>Normal proteinuria</td>
<td>36 (62.1)</td>
<td>14 (24.1)</td>
<td>8 (13.8)</td>
<td>4.852</td>
<td>0.088</td>
<td>86 (74.1)</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>17 (42.5)</td>
<td>13 (32.5)</td>
<td>10 (25.0)</td>
<td>3.788</td>
<td>0.150</td>
<td>47 (58.8)</td>
</tr>
<tr>
<td>Massive proteinuria</td>
<td>2 (20.0)</td>
<td>3 (30.0)</td>
<td>5 (50.0)</td>
<td>10.191</td>
<td>0.006</td>
<td>7 (35.0)</td>
</tr>
</tbody>
</table>

Genotype and allele comparison of Fn Msp iv between normal and abnormal proteinuria groups

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Normal proteinuria, n (%)</th>
<th>Abnormal proteinuria, n (%)</th>
<th>OR (95% CI)</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>36 (62.1)</td>
<td>19 (38.0)</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD</td>
<td>14 (24.1)</td>
<td>16 (32.0)</td>
<td>2.165 (0.874-5.366)</td>
<td>2.829</td>
<td>0.110</td>
</tr>
<tr>
<td>DD</td>
<td>8 (13.8)</td>
<td>15 (30.0)</td>
<td>3.553 (1.278-9.875)</td>
<td>6.205</td>
<td>0.023</td>
</tr>
<tr>
<td>C</td>
<td>86 (74.1)</td>
<td>54 (54.0)</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>30 (29.3)</td>
<td>46 (46.0)</td>
<td>2.442 (1.378-4.327)</td>
<td>9.550</td>
<td>0.003</td>
</tr>
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</table>

Comparison of genotypes and alleles of patients with DN in late-onset or free of DN group and early-onset group

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Late-onset DN, n (%)</th>
<th>Early-onset DN, n (%)</th>
<th>OR (95% CI)</th>
<th>( \chi^2 )</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>20 (66.7)</td>
<td>6 (30.0)</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD</td>
<td>6 (20.0)</td>
<td>5 (25.0)</td>
<td>2.778 (0.622-12.411)</td>
<td>1.853</td>
<td>0.173</td>
</tr>
<tr>
<td>DD</td>
<td>4 (13.3)</td>
<td>9 (45.0)</td>
<td>7.500 (1.691-33.272)</td>
<td>7.800</td>
<td>0.005</td>
</tr>
<tr>
<td>C</td>
<td>46 (76.7)</td>
<td>17 (42.5)</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>14 (23.3)</td>
<td>23 (57.5)</td>
<td>4.445 (1.869-10.574)</td>
<td>12.019</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Discussion

Epidemiology studies have shown that the incidence of some diseases presents significant differences in various regions [15]. In addition to the vital effects of environmental factors, heterogeneity of genetic backgrounds corresponding with certain races can also cause variance in diseases susceptibility [16]. Study on genetic polymorphisms in human is helpful to make clear the function of different genotypes, which also contributes to the early diagnosis and fine treatment of disease [17].
molecular weight, can be observed when angiotensin II (AngII) stimulates proliferation and sclerosis of mesangial cells (MSCs). And AngRem104 mRNA, involving up-regulation of Fn, has been found to express in mesangial and renal tubular epithelial cells [18-22]. Fn is a kind of ubiquitous non-collagen glycoprotein with various biological functions, and it mainly distributed in basement membranes adjacent to inside or outside elastic layers, smooth muscle cells, and areolar tissues [14]. Fn gene promotes stromal cells growth, division and proliferation of fibrocytes. In addition, some studies have demonstrated that Fn gene was closely related to pulmonary fibrosis [23, 24]. Therefore, further investigation the relationship between Fn polymorphism and DN plays an important role in the treatment of DN.

Our study analyzed Fn Msp iv genotypes and clinicopathologic features of patients with DN. The results demonstrated that genotype and allele distribution in massive proteinuria group showed great differences, compared with those of control group (P = 0.006, P = 0.004). The further analysis showed that DD genotype and D allele appeared to be a risk factor for abnormal proteinuria. Then, we analyzed the association of genotype distribution of Fn Msp iv and clinicopathologic stages of DN. The result indicated that DD genotype showed greater effect on the occurrence of early-onset DN. In addition, the D allele was significantly associated with the susceptibility of early-onset DN.

Our study demonstrated that there existed significant association of Fn Msp iv polymorphism and DN susceptibility. However, the sample size was relatively small. The effects of Fn genetic mutations in the pathogenesis of DN need to be further investigated.

Disclosure of conflict of interest

None.

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