

Original Article

Investigation on the association between interleukin-10 -592C/A, -819C/T and -1082A/G gene polymorphisms and development of diabetic nephropathy

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Abstract: We conducted a case-control study to investigate the association between interleukin (IL)-10-592C/A, -819C/T and -1082A/G polymorphisms and susceptibility to diabetic nephropathy. A hospital-based case-control study was taken in our study. A total of 172 patients with proven type 2 diabetes mellitus and 344 controls were recruited from the First Affiliated Hospital of Xinxiang Medical University between March 2012 and October 2014. Genotyping of *IL-10* -592C/A, -819C/T and -1082A/G polymorphisms was done by PCR-RFLP methods. By the χ^2 test, the distributions of the GG, GA and AA genotypes in *IL-10* -1082A/G were significantly different between patients with diabetic nephropathy and control subjects ($\chi^2 = 8.09$, $P = 0.02$). By conditional logistic regression analysis, we found that the AA genotype of *IL-10* -1082A/G was associated with an elevated risk of diabetic nephropathy compared to the GG genotype in codominant model, and the adjusted OR (95% CI) was 2.38 (1.23-4.57). In dominant model, the GA+AA genotype was associated with a significantly increased risk of diabetic nephropathy compared to the GG genotype in dominant model (OR = 1.47, 95% CI = 1.05-2.16). In recessive model, the AA genotype could influence the susceptibility to diabetic nephropathy when compared with the GG+GA in recessive model (OR = 2.08, 95% CI = 1.12-3.85). In conclusion, we suggested that *IL-10* -1082A/G gene polymorphism was correlated with development of diabetic nephropathy, but no association was observed between *IL-10* -819T/C and -592A/C and risk of diabetic nephropathy.

Keywords: Diabetic nephropathy, *IL-10* -1082A/G, *IL-10* -819T/C, *IL-10* -592A/C, polymorphism

Introduction

Diabetic nephropathy is a major cause of end-stage renal disease (ESRD) and high mortality in diabetic patients [1]. The etiology of diabetic nephropathy is not well understood, and the process of diabetic nephropathy is caused by many environmental factors, such as high blood pressure, high glomerular filtration rate, glycemic control and ethnicities [2]. However, not all of the individuals who expose to similar risk factors would suffer from diabetic nephropathy, which suggests that genetic factors have an important role in the susceptibility to diabetic nephropathy.

Interleukin-10 (*IL-10*) is an immunoregulatory cytokine and is produced by Th2 cells, and this gene plays an important role in regulating T cells, and monocytes/macrophages. It is well

known that the encoding gene of *IL-10* is located on chromosome 1 (1q31-1q32), and is an anti-inflammatory cytokine, which could inhibit the synthesis of cytokines, such as *IL-6*, *IL-1 β* , *IL-1 α* , and TNF- α in activated macrophage and IFN γ by T cells [3]. Previous study has reported that increased concentration of circulating *IL-10* was associated with the development of diabetes mellitus patients with diabetic nephropathy [4]. Another study has reported that alterations of *IL-10* levels are associated with the extent of renal damage in diabetic nephropathy [5]. These studies suggest that *IL-10* may contribute to the pathogenesis of diabetic nephropathy. Three common SNPs are observed in *IL-10* genes, including *IL-10* -592C/A, -819C/T and -1082A/G. Previous studies have reported the *IL-10* -592C/A, -819C/T and -1082A/G polymorphisms and development of diabetic nephropathy, but the results are inconclusive [6-8]. In our

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Table 1. Demographic and clinical characteristics of patients with diabetic nephropathy and control subjects

| Variables | Patients | % | Controls | % | t test or χ^2 test | P value |
|-----------------------------|----------|------------|----------|-----------|-------------------------|---------|
| Age, years | | | | | | |
| <55 | 80 | 46.51 | 162 | 47.09 | | |
| ≥55 | 92 | 53.49 | 182 | 52.91 | 0.02 | 0.90 |
| Gender | | | | | | |
| Females | 108 | 62.79 | 216 | 62.79 | | |
| Males | 64 | 37.21 | 128 | 37.21 | 0.00 | 1.00 |
| BMI, kg/m ² | | | | | | |
| <24 | 63 | 36.63 | 232 | 67.44 | | |
| ≥24 | 109 | 63.37 | 112 | 32.56 | 44.46 | <0.001 |
| Hypertension | | | | | | |
| Yes | 61 | 35.47 | 218 | 63.37 | | |
| No | 111 | 64.53 | 126 | 36.63 | 35.96 | <0.001 |
| Triglyceride | | 1.79±0.07 | | 1.42±0.05 | 61.87 | <0.001 |
| Total cholesterol | | 4.79±0.06 | | 5.11±0.08 | 50.90 | <0.001 |
| High density lipoprotein | | 1.17±0.03 | | 1.24±0.05 | 19.81 | <0.001 |
| Low density lipoprotein | | 3.12±0.07 | | 3.24±0.08 | 17.49 | <0.001 |
| Creatinine | | 1.73±0.74 | | 0.92±0.13 | 19.72 | <0.001 |
| Duration of diabetes, years | | 11.42±3.10 | | | | |

study, we conducted a case-control study to investigate the association between *IL-10* -592C/A, -819C/T and -1082A/G polymorphisms and susceptibility to diabetic nephropathy.

Materials and methods

Study subjects

A hospital-based case-control study was taken in our study. A total of one hundred and seventy two patients with proven type 2 diabetes mellitus were recruited from the First Affiliated Hospital of Xinxiang Medical University between March 2012 and October 2014. The diagnosis of type 2 diabetes mellitus was based on the WHO criteria in 1999 [9]. The nephropathy was diagnosed in diabetic patients according to proteinuria of at least 500 mg/24 h and glomerular filtration rates (GFR) of less than 25 ml/min.

Three hundred and forty four control subjects free of diabetes and nephropathy were collected from individuals who underwent a regular health examination at the examination center of our hospital during the same period of time. Two control subjects were age- and gender-matched with one patient. Control subjects who had a history of type 2 diabetes mellitus

and nephropathy were excluded from the present study.

A standardized questionnaire was used to investigate the demographic characteristics of patients with diabetic nephropathy and control subjects, including age, sex, diabetic duration and body mass index (BMI). The clinical data were collected from medical records, including systolic and diastolic blood pressure, the levels of total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C) and low density lipoprotein (LDL-C) and creatinine were determined. Signed informed consent forms were obtained from all the study subjects prior to their participation in the present study. The protocol of our study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University.

Genotyping

Each patient with diabetic nephropathy and control subject was asked to provide 5 ml blood sample, and the blood sample was collected in EDTA-containing tubes, and stored at -20°C until use. Genomic DNA was isolated from blood sample using the QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA). Genotyping of *IL-10* -592C/A, -819C/T and -1082A/G polymor-

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Table 2. Distribution of *IL-10* -1082A/G, -819T/C, and -592A/C gene polymorphisms

| SNP | Patients | % | Controls | % | P for HWE | χ^2 value | P value | Minor allele frequency | |
|-----------------|----------|-------|----------|-------|-----------|----------------|---------|------------------------|-------------|
| | | | | | | | | In database | In controls |
| -1082A/G | | | | | | | | | |
| GG | 66 | 38.37 | 163 | 47.38 | 0.28 | 8.09 | 0.02 | 0.2722 | 0.3009 |
| GA | 80 | 46.51 | 153 | 44.48 | | | | | |
| AA | 26 | 15.12 | 27 | 7.85 | | | | | |
| -819T/C | | | | | | | | | |
| TT | 57 | 33.14 | 127 | 36.92 | 0.38 | 0.53 | 0.77 | 0.4347 | 0.4128 |
| TC | 77 | 44.77 | 150 | 43.60 | | | | | |
| CC | 38 | 22.09 | 67 | 19.48 | | | | | |
| -592A/C | | | | | | | | | |
| AA | 67 | 38.95 | 132 | 38.37 | 0.32 | 1.18 | 0.55 | 0.4349 | 0.3910 |
| AC | 79 | 45.93 | 155 | 45.06 | | | | | |
| CC | 26 | 15.12 | 57 | 16.57 | | | | | |

Table 3. Association between *IL-10* -1082A/G, -819T/C, and -592A/C gene polymorphisms and risk of diabetic nephropathy

| Model | IL-10 | Patients | % | Controls | % | OR (95% CI) ¹ | P value |
|-----------------|-------|----------|-------|----------|-------|--------------------------|---------|
| -1082A/G | | | | | | | |
| Codominant | GG | 66 | 38.37 | 163 | 47.38 | 1.0 (Ref.) | - |
| | GA | 80 | 46.51 | 153 | 44.48 | 1.29 (0.85-1.95) | 0.2 |
| | AA | 26 | 15.12 | 27 | 7.85 | 2.38 (1.23-4.57) | 0.05 |
| Dominant | GG | 66 | 38.37 | 163 | 47.38 | 1.0 (Ref.) | - |
| | GA+AA | 106 | 61.63 | 180 | 52.33 | 1.47 (1.05-2.16) | 0.04 |
| Recessive | GG+GA | 146 | 84.88 | 316 | 91.86 | 1.0 (Ref.) | - |
| | AA | 26 | 15.12 | 27 | 7.85 | 2.08 (1.12-3.85) | 0.01 |
| -819T/C | | | | | | | |
| Codominant | TT | 57 | 33.14 | 127 | 36.92 | 1.0 (Ref.) | - |
| | TC | 77 | 44.77 | 150 | 43.60 | 1.14 (0.74-1.77) | 0.53 |
| | CC | 38 | 22.09 | 67 | 19.48 | 1.26 (0.74-2.16) | 0.36 |
| Dominant | TT | 57 | 33.14 | 127 | 36.92 | 1.0 (Ref.) | - |
| | TC+CC | 115 | 66.86 | 217 | 63.08 | 1.18 (0.79-1.77) | 0.40 |
| Recessive | TT+TC | 134 | 77.91 | 277 | 80.52 | 1.0 (Ref.) | - |
| | CC | 38 | 22.09 | 67 | 19.48 | 1.17 (0.73-1.87) | 0.49 |
| -592A/C | | | | | | | |
| Codominant | AA | 67 | 38.95 | 132 | 38.37 | 1.0 (Ref.) | - |
| | AC | 79 | 45.93 | 155 | 45.06 | 1.01 (0.66-1.53) | 0.98 |
| | CC | 26 | 15.12 | 57 | 16.57 | 0.89 (0.50-1.61) | 0.7 |
| Dominant | AA | 67 | 38.95 | 132 | 38.37 | 1.0 (Ref.) | - |
| | AC+CC | 105 | 61.05 | 212 | 61.63 | 0.98 (0.66-1.45) | 0.9 |
| Recessive | AA+AC | 146 | 84.88 | 287 | 83.43 | 1.0 (Ref.) | - |
| | CC | 26 | 15.12 | 57 | 16.57 | 0.90 (0.52-1.52) | 0.67 |

¹Adjusted for age, gender, BMI, hypertension, triglyceride, total cholesterol, high density lipoprotein, low density lipoprotein and creatinine.

phisms was done by done by PCR-RFLP methods. Probes and primers for *IL-10* -592C/A, -819C/T and -1082A/G were designed by Se-

quenom Assay Design 3.1 software (Sequenom®) according to the manufacturer's instructions. The PCR conditions were set as follows:

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95°C for 5 min, 30 cycles of 95°C for 30 s, 63°C for 30 s, and 72°C for 30 s and a final extension step of 72°C for 10 min. Quality control was ensured by blind genotyping of the 10% of the subject samples.

Statistical analysis

Statistical analysis was done using statistical package SPSS 18.0 package (SPSS Inc., Chicago, IL, USA). Differences between patients with diabetic nephropathy and control subjects were analyzed by independent sample t-test and χ^2 test. Association between *IL-10* polymorphisms and risk of diabetic nephropathy were calculated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. A *P*-value less than 0.05 were considered statistically significant.

Results

Because of matching by age and gender, there was no statistical analysis difference between patients with diabetic nephropathy and control subjects in terms of age and gender (**Table 1**). By χ^2 test, patients with diabetic nephropathy were more likely to be suffer from hypertension ($\chi^2 = 35.96, P < 0.001$), have higher BMI ($\chi^2 = 44.46, P < 0.001$), higher triglyceride ($t = 61.87, P < 0.001$), total cholesterol ($t = 50.90, P < 0.001$), high density lipoprotein ($t = 19.81, P < 0.001$), low density lipoprotein ($t = 17.49, P < 0.001$) and creatinine ($t = 19.72, P < 0.001$) when compared with controls.

The genotype distributions of *IL-10* -1082A/G, -819T/C, and -592A/C genes confirmed with the Hardy-Weinberg equilibrium in the control subjects (**Table 2**), and the *P* values were 0.28, 0.38 and 0.32, respectively. By the χ^2 test, the distributions of the GG, GA and AA genotypes in *IL-10* -1082A/G were significantly different between patients with diabetic nephropathy and control subjects ($\chi^2 = 8.09, P = 0.02$). The minor allele frequency in controls was similar with the distributions in dbSNP database (<http://www.ncbi.nlm.nih.gov/snp/>).

By conditional logistic regression analysis, we found that the AA genotype of *IL-10* -1082A/G was associated with an elevated risk of diabetic nephropathy compared to the GG genotype in codominant model, and the adjusted OR

(95% CI) was 2.38 (1.23-4.57) (**Table 3**). In dominant model, the GA+AA genotype was associated with a significantly increased risk of diabetic nephropathy compared to the GG genotype in dominant model (OR = 1.47, 95% CI = 1.05-2.16). In recessive model, the AA genotype could influence the susceptibility to diabetic nephropathy when compared with the GG+GA in recessive model (OR = 2.08, 95% CI = 1.12-3.85). However, we did not find that the *IL-10* -819T/C and -592A/C could influence the development of diabetic nephropathy.

Discussion

It is reported that individuals could not develop the same type of diseases even when they expose to similar risk factors of diseases, and thus we could hypothesize that genetic variations may contribute to the development of diabetic nephropathy. We conducted a case-control study to investigate the association between *IL-10* -1082A/G, -819T/C, and -592A/C gene polymorphisms and development of diabetic nephropathy, and we found that *IL-10* -1082A/G gene polymorphism was associated with susceptibility to diabetic nephropathy in codominant, dominant and recessive models.

Increasing evidences suggested that pathogenesis of diabetes mellitus was correlated with the presence of a chronic low-grade inflammatory state and the activation of the innate immune system [9]. Moreover, it is reported that inflammatory cytokines are involved in the development of microvascular diabetic complications, including diabetic nephropathy [11, 12]. *IL-10* was a candidate gene in the pathophysiologic mechanism of auto-immune/inflammatory disease, because it could regulate both cellular and humoral immunity. Previous study has suggested that the *IL-10* elevated in the sera of type 2 diabetes mellitus patients with diabetic nephropathy [5]. Thus, high expression of *IL-10* might be associated with an increased risk of diabetic nephropathy.

Several previous studies have reported the association between *IL-10* gene polymorphisms and development of diabetic nephropathy, but the results are inconsistent [6-8, 13-16]. Babel et al. conducted a case-control study in German, and they reported that *IL-10* -1082A/G may increase the risk of patients with type 2 diabetes or glomerulonephritis [14]. Another

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two studies in a Tunisian population, they reported that *IL-10* -819T/C was associated with increased risk of diabetic nephropathy, but no association was found between *IL-10* -1082A/G and development of diabetic nephropathy [6, 15]. Another study also conducted in a Taiwanese population, and they found that *IL-10* -592A/C may influence products of *IL-10*, and it is an indicator of nephropathy risk in Taiwanese type 2 diabetes mellitus [7]. Arababadi et al. found that *IL-10* -592A/C polymorphism has an effect in the pathogenesis of diabetes with nephropathy [16]. Erdogan et al. conducted a study in a Turkish population, and they reported that *IL-10* -1082A/G is not correlated with the development of diabetic nephropathy [8]. In our study, we found that *IL-10* -1082A/G gene polymorphism was associated with susceptibility to diabetic nephropathy, but no association between *IL-10* -819T/C, and -592A/C and risk of diabetic nephropathy. The conflicting results about the role of *IL-10* -1082A/G, -819T/C, and -592A/C gene polymorphisms in diabetic nephropathy may be due to different ethnicities, sample size and study design.

Two limitations in our study should be taken into consideration. First, study subjects were selected from only one hospital, which would cause selection bias in our study. However, the genotype distributions of *IL-10* -1082A/G, -819T/C, and -592A/C are in line with the Hardy-Weinberg equilibrium in controls, which suggests that our population may represent the general population. Second, the sample size of our study is relatively small, which could reduce the statistical power to find differences between groups. Therefore, further studies with participants from multiple locations and a larger sample size are needed to confirm our results.

In conclusion, we suggest that *IL-10* -1082A/G gene polymorphism is correlated with development of diabetic nephropathy, but no association was observed between *IL-10* -819T/C and -592A/C and risk of diabetic nephropathy. Further large sample size and multicenter studies are greatly needed to confirm their association.

Disclosure of conflict of interest

None.

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