Research Article

Association of Interleukin-1 Beta and Interleukin-1 Receptor Antagonist Gene Polymorphisms and Plasma Levels with Diabetic Nephropathy

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Received 31 January 2022; Revised 18 April 2022; Accepted 7 May 2022; Published 25 May 2022

Academic Editor: Wan Amir Nizam Wan Ahmad

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Objective. We investigated the relationships between interleukin- (IL-) 1β and IL-1 receptor antagonist (IL-1Ra) gene polymorphism and plasma levels in patients with diabetic nephropathy (DN).

Methods. The genotype and allele frequency distribution of IL-1β and IL-1Ra in 61 patients with DN and 48 healthy controls (HCs) were determined by kompetitive allele-specific PCR (KASP), and the plasma concentrations of IL-1β and IL-1Ra in DN patients and HCs were measured by enzyme-linked immunosorbent assays (ELISA).

Results. Significant differences were detected in the distribution of IL-1β (−511C/T) genotype and allele frequencies between the DN and HC groups (P<0.05), with the T genotype being more frequent in DN patients than HCs (OR = 2.84, 95% CI: 1.489–5.416). The IL-1β (+3953C/T) and IL-1Ra (+8006C/T) genotypes and allele frequencies were not significantly different between the two groups (P>0.05). The plasma IL-1β level was significantly higher (P<0.01), while the plasma IL-1Ra concentration was significantly lower in the DN group than the HC group (P<0.05). Furthermore, the plasma IL-1β level was significantly different between IL-1β (−511C/T) locus variants (P<0.05). The IL-1β (−511C/T) gene polymorphism was significantly associated with DN risk in the population of northern Guangxi, China, and the T allele may be responsible for genetic susceptibility to DN.

Conclusion. The IL-1β (−511C/T) gene polymorphism was significantly associated with DN risk in the population of northern Guangxi, China, and the T allele may be responsible for genetic susceptibility to DN.

1. Introduction

Diabetic nephropathy (DN) is one of the most common and severe microvascular complications of type 2 diabetes mellitus (T2DM), and it may progress to end-stage renal disease (ESRD) [1]. To date, the mechanisms underlying the pathogenesis of DN have remained unclear; however, genetic susceptibility and inflammation have been suggested to be critical factors in the development and progression of DN [2].

The interleukin- (IL-) 1 family of cytokines consists of eleven members: IL-1α, IL-1β, IL-1Ra, IL-18, IL-33, IL-36Ra, IL-36α, IL-36β, IL-36y, IL-37, and IL-38, which are encoded at three separate loci: two on chromosomes 11 and 9, with the other nine coding genes clustered on the short arm of human chromosome 2 [3, 4]. The main
functions of IL-1β are proinflammatory; they include the modulation of helper T-cell 17 (Th17) responses and tissue remodeling in many inflammatory and autoimmune diseases [5]. In humans, IL-1β has been identified as an important mediator of the inflammatory response [6]. Patients with renal disease have elevated circulating levels of IL-1β [7]. Furthermore, IL-1 receptor antagonist (IL-1Ra) is a cytokine for which the only known action is the competitive inhibition of IL-1 binding and thus modulation of the activity of IL-1β [8].

In patients with insulin resistance, IL-1 cytokine family and cytokine receptors promote (IL-1β)/inhibit (IL-1Ra) macrophage activation [9]. IL-1β and IL-1Ra are important mediators of chronic inflammation and tissue damage in multiple organs [10] and are also closely related to T2DM and its complications [11]. Results in a gerbil model showed that high concentrations of glucose induce IL-1β production and secretion from human β-cells, leading to the upregulation of Fas receptor, NF-kB activation, β-cell apoptosis, and renal dysfunction [12]. Similarly, in a mouse model of DN, IL-1β caused renal inflammation and deterioration of renal function [13]. Moreover, IL-1Ra may also contribute to renal inflammation in DN with antagonizing effects [14]. Salti et al. [15] suggested that IL-1Ra treatment can prevent the progression and even reverse the evolution of DN in animal models. With increasing understanding, IL-1 family polymorphisms have been used to explain some inherited traits and susceptibility to diabetic disease [11, 16]. A survey in South Korea showed that the IL1B2 and IL1RN*+2 genotypes of the IL-1 gene cluster were associated with DN in Korean patients with T2DM and additionally that carrying these alleles may increase the risk of renal failure [17].

There have been no previous reports regarding the relations between IL-1 polymorphisms and susceptibility to DN in the population of northern Guangxi, China. Here, we investigated the correlations of IL-1β in the population of northern Guangxi, China. Here, we investigated the correlations of IL-1β and IL-1Ra with DN in a cohort from northern Guangxi. We used the competitive allele-specific PCR (KASP) technique to investigate the relations between IL-1β and IL-Ra gene polymorphisms and their plasma levels in DN patients and healthy controls (HCs).

2. Materials and Methods

2.1. Study Participants. The expression level of IL-1β in healthy people was 11.7 pg/mL; 48 HCs were collected in this study. PASS 15.0.5 was used to estimate the sample size required for the DN group. The test level α was set as 0.5, and the degree of certainty (1 – β) was set as 90%; thus, at least 50 cases were needed to calculate the DN group. This case-control study included 61 patients diagnosed with DN at The Affiliated Hospital of Guilin Medical College (Guangxi, China) between January 2020 and July 2020 (34 males and 27 females). A diagnosis of T2DM was made according to the diagnostic and classification criteria for diabetes established by the World Health Organization (WHO) in 1998 [18]: a fasting blood glucose level (FPG) ≥ 7.0 mmol/L and/or oral glucose tolerance test (OGTT) 2 h postprandial blood glucose level ≥ 11.1 mmol/L, a confirmed diagnosis of T2DM, and currently taking oral hypoglycemic drugs or insulin injection treatment. The inclusion criteria for DN were patient state consistent with the diagnosis of diabetes and one of the following conditions: urinary albumin excretion rate (UAER) > 30 mg/24 h at least twice, urinary protein level > 0.5 g/24 h, and serum creatinine > 167 μmol/L. Patients with other diseases that cause proteinuria and renal insufficiency were excluded. During the same period, 48 healthy volunteers were recruited as controls (23 males and 25 females). Individuals with a previous history of diabetes, obesity, hyperlipidemia, arterial hypertension, coronary heart disease, hepatitis, infections, and other diseases were excluded from the study. Subjects who had also recently taken antibiotics and other drugs were excluded. All subjects were genetically unrelated individuals from northern Guangxi, and there were no statistically significant differences in sex, age, or other demographic characteristics between the groups (P > 0.05).

The present study was conducted in accordance with the principles of the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the Affiliated Hospital of Guilin Medical College. All subjects agreed to have blood withdrawn and provided signed informed consent.

2.2. Blood Collection and Processing

2.2.1. Blood Specimens. Venous blood was extracted from fasted subjects into biochemical anticoagulant tubes. After centrifugation at 1000 × g for 10 min at room temperature, the plasma supernatant was collected and stored at −80°C.

2.2.2. DNA Isolation. Peripheral venous blood (2 mL) was used for extraction of genomic DNA using a Whole Blood Genomic DNA Extraction Kit (Beijing Adlai Biotechnology Co., Ltd., Beijing, China). Briefly, 900 μL of 1 x red cell lysate was pipetted into a 1.5 mL centrifuge tube, followed by the addition of 300 μL of anticoagulant, and mixed well. The mixture was centrifuged at 12,000 rpm at 4°C for 20 s to obtain the leukocyte precipitate; then we added 300 μL of cell lysate to the resuspended leukocytes. This was followed by the addition of 100 μL of protein precipitation solution and vortex mixing. The mixture was centrifuged at 13,000 rpm for 5 min, and the supernatant was carefully transferred to a fresh 1.5 mL centrifuge tube. Then, 300 μL of isopropanol was added followed by mixing and centrifugation at 12,000 rpm for 1 min. The supernatant was discarded and 1 mL of 70% ethanol was added, followed by centrifugation at 12,000 rpm for 1 min. The resultant precipitate was then dried. The DNA precipitate was solubilized by mixing with 100 μL of DNA lysis solution and then stored at −20°C.

2.2.3. Genotyping. The primers for KASP were designed using Primer5 software (https://primer5.ut.ee/) and synthesized by Shanghai Bioengineering Co., Ltd. (Shanghai, China). The single nucleotide polymorphism (SNP) of the IL-1β (−511C/T) rs16944, the SNP of the IL-1β (−3953C/T) rs1143634, and the SNP of the IL-1Ra (+8006C/T) rs419598 were investigated. Target SNPs were analyzed by real-time PCR with the following primers: (1) rs16944F1: 5′-GAAGGTGACCAAGTTTCTCAG-3′; rs16944R1: 5′-GGGTTTGAGGAGAGAAAAGTTTG-3′; rs1143634F1: 5′-CGGCTGCTGATGAGAACTTG-3′; rs1143634R1: 5′-GGTGGATTTTGGTGTTCCTT-3′; rs419598F1: 5′-CTTTGCAATCTTTTTGCTTTCTC-3′; rs419598R1: 5′-AGTTTTTTTTTATTTTTATTTTTTTT-3′.
ATGCTTGGGTGCTGTCTGCTCCTCA-3'; rs16944F2: 5'-GAAGGTCGGAGTCAACGGATTGGAACACAGATGAT TTGGTCCTTGCAA-3'; rs16944F3: 5'-GAAGGTGACCAAGTTCATGCTAGGAA-3'; rs16944F1: 5'-GAAGGTGACCAAGTTCATGCTAGGAA-3'.

3.1. Main Characteristics and Genotype Distributions of SNPs

Table 1: Baseline characteristics.

| Parameter | DN   | HC   |
|-----------|------|------|
| Cases     | 61   | 48   |
| Gender (M/F) | 34/27 | 23/25 |
| Age (years)  | 61.39 ± 11.25 | 57.93 ± 9.84 |
| Height (cm)  | 165.57 ± 12.61 | 166.91 ± 28.39 |
| BMI (kg/m²)  | 23.98 ± 5.00 | 23.88 ± 2.15 |
| SBP (mmHg)   | 146.15 ± 22.82 | 128.73 ± 17.15 |
| DBP (mmHg)   | 87.41 ± 14.51 | 75.16 ± 10.43 |
| HbA1c (%)    | 8.83 ± 2.51 | 5.56 ± 0.46 |
| UAER (mg/24h) | 2065.71 ± 2269.48 | — |
| BUN (mmol/L) | 8.48 ± 6.51 | 4.56 ± 1.09 |
| Cr (μmol/L)  | 177.13 ± 215.45 | 70.81 ± 17.11 |
| TG (mmol/L)  | 2.36 ± 2.11 | 1.69 ± 0.86 |
| TC (mmol/L)  | 4.32 ± 1.36 | 5.10 ± 1.00 |

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; UAER: urinary albumin excretion rate; BUN: blood urea nitrogen; Cr: creatinine; TG: triglyceride; TC: total cholesterol. Statistical evaluation: t-test; *P < 0.05 versus the HC group.

3.2. Associations of IL-1β and IL-1Ra Genotype Distributions and Allele Frequencies in DN Patients. Our results show that the numbers of individuals of TT, TC, and CC genotypes of rs16944 (−511C/T) were 46 (75.4%), 11 (18%), and 4 (6.6%) in the DN group and 22 (45.8%), 19 (39.6%), and 7 (14.6%) in the HC group, respectively. Moreover, the frequencies of the T allele of the IL-1β gene rs16944 in the DN and HC groups were 84.4% and 65.6%, respectively. Statistical analysis showed that there were significant differences between the two groups in the distribution of IL-1β (−511C/T) genotypes (χ² = 10.014, P < 0.05) and the allele frequencies of IL-1β (−511C/T) (χ² = 10.456, P < 0.01). In addition, an analysis of the allele frequencies showed a 1.84-fold higher frequency for genotypes carrying the T allele at IL-1β (−511C/T) compared to those carrying the C allele (OR = 2.84, 95% CI: 1.489–5.416) (Table 3). However, the genotype distribution and allele frequencies of IL-1β (+3953C/T) and IL-1Ra (+8006C/T) were not significantly different between the two groups (Table 3).

3.3. Evaluation of Plasma IL-1β and IL-1Ra Levels. The plasma IL-1β concentration was significantly higher in the DN group than the HC group (18.24 ± 1.88 vs. 11.63 ± 0.72 pg/mL, respectively, P < 0.01). In contrast, the plasma IL-1Ra levels were lower in the DN group than in the HC group (1432.20 ± 168 vs. 2026.31 ± 168.6 pg/mL, respectively, P < 0.05) (Figure 1).
3.4. Evaluation of Plasma IL-1β and IL-1Ra Levels with Different Genotypes. We determined the correlations of plasma IL-1β concentrations with TT, TC, and CC genotypes in 109 participants to explore the relations between each allele of the IL-1β (-511C/T) polymorphism and plasma levels of IL-1β and IL-1Ra. The plasma IL-1β concentrations were 16.95 ± 1.54 pg/mL for the TT genotype, 10.21 ± 0.98 pg/mL for the TC genotype, and 19.27 ± 4.85 pg/mL for the CC genotype. Thus, the plasma IL-1β concentration was significantly higher in subjects with the TT genotype compared with the TC genotype. In addition, the plasma IL-1β levels in subjects with TT, TC, and CC genotypes in the DN group were 19.45 ± 1.46, 33.19 ± 10.02 pg/mL, respectively, indicating that plasma IL-1β levels were significantly higher in homozygous compared to heterozygous DN patients. The plasma IL-1Ra levels did not differ significantly between genotypes (Figure 2). Moreover, there were no significant differences

| Genotype | Group  | N   | TT        | Genotype (%) | CC        | P          | \(\chi^2\)   |
|----------|--------|-----|-----------|--------------|-----------|------------|-------------|
| IL-1β (-511C/T) | DN      | 61  | 46 (75.4) | 11 (18.0)    | 4 (6.6)   | \(\chi^2 = 1.612\) | 0.45         |
|          | HC      | 48  | 22 (45.8) | 19 (39.6)    | 7 (14.6)  | \(\chi^2 = 0.309\) | 0.85         |
| IL-1β (+3953C/T) | DN      | 61  | 13 (21.3) | 30 (49.2)    | 18 (29.5) | \(\chi^2 = 0.54\)  | 0.05         |
|          | HC      | 48  | 10 (20.8) | 24 (50.0)    | 14 (29.2) | \(\chi^2 = 6.443\) | 0.009        |
| IL-1Ra (+8006C/T) | DN      | 61  | 1 (1.6)   | 10 (16.4)    | 50 (82.0) | \(\chi^2 = 0.304\) | 1.00         |
|          | HC      | 48  | 1 (2.1)   | 8 (16.7)     | 39 (81.2) | \(\chi^2 = 0.318\) | 1.00         |

| Genotype or allele | DN (N = 61) | HC (N = 48) | \(\chi^2\) | \(P\) | OR (95% CI) |
|--------------------|-------------|-------------|-------------|------|-------------|
| IL-1β (-511)       |             |             |             |      |             |
| TT                 | 46 (75.4)   | 22 (45.8)   | 10.014      | 0.01*| 3.66 (0.968–13.827) |
| TC                 | 11 (18.0)   | 19 (39.6)   | 1.01 (0.683–1.473) | 1.00 |
| CC                 | 4 (6.6)     | 7 (14.6)    | 1.00        |      |             |
| T                  | 103 (84.4)  | 63 (65.6)   | 10.465      | <0.01**| 2.84 (1.489–5.416) |
| C                  | 19 (15.6)   | 33 (34.4)   | 1.00        |      |             |
| IL-1β (+3953)      |             |             |             |      |             |
| TT                 | 16 (26.2)   | 16 (33.3)   | 2.510       | 0.29 | 1.91 (0.755–4.802) |
| TC                 | 24 (39.3)   | 12 (25.0)   | 1.00        |      |             |
| CC                 | 21 (34.4)   | 20 (41.7)   | 1.00        |      |             |
| T                  | 56 (45.9)   | 44 (45.8)   | 0.000       | 0.99 | 1.00 (0.586–1.715) |
| C                  | 66 (54.1)   | 52 (54.2)   | 1.00        |      |             |
| IL-1Ra (+8006)     |             |             |             |      |             |
| TT                 | 1 (1.6)     | 1 (2.1)     | 0.318       | 1.00 | 0.98 (0.352–2.703) |
| TC                 | 10 (16.4)   | 8 (16.7)    | 1.00        |      |             |
| CC                 | 50 (82.0)   | 39 (81.2)   | 1.00        |      |             |
| T                  | 12 (9.8)    | 10 (10.4)   | 0.020       | 0.89 | 0.94 (0.387–2.274) |
| C                  | 110 (90.2)  | 86 (89.6)   | 1.00        |      |             |

* indicates \(P < 0.05\); ** indicates \(P < 0.01\).
in plasma IL-1β levels between genotypes for the IL-1β (+3953C/T) locus polymorphism and plasma IL-1Ra levels between genotypes for the IL-1Ra (+8006C/T) locus polymorphism (data not shown).

4. Discussion

In this study, we examined the polymorphisms of IL-1β and IL-1Ra genes in 109 individuals (61 DN patients, 48 HCs). The results show that the IL-1β (−511C/T) rs16944 distributions of genotype and allele frequencies were significantly different between the two groups, with the frequency of DN patients with T allele being significantly higher than in the HC group. Furthermore, no significant associations were observed between IL-1β (+3953C/T) rs1143634 or IL-1Ra (+8006C/T) rs419598 and DN. To our knowledge, this is the first study to investigate the association of IL-1β and IL-1Ra SNPs with the risk of DN in a population from northern Guangxi.

The anti-inflammatory genes IL-1β and IL-1Ra were identified on the long arm of human chromosome 2 [19].
The IL-1β gene has two C>T polymorphic sites at promoter −511 and exon +3953, and the IL-1Ra gene has a variable copy number of an 86-bp sequence in intron 2 [20, 21]. Our results are consistent with those of previous studies. An early study demonstrated that the IL-1β (−511C/T) polymorphism was associated with DN and that the T allele was more common in patients with DN than in healthy individuals [22]. An additional logistic regression analysis indicated that the IL-1β (−511C/T) SNP had an impact on the prevalence of ESRD [23]. A subsequent meta-analysis of 30 published trials concluded that IL-1B −511C/T polymorphism may influence predisposition to DN in both Caucasians and Asians [24].

We further investigated the relationship between IL-1β and IL-1Ra gene polymorphisms and plasma IL-1β and IL-1Ra concentrations in DN patients and HCs. Our results show that the plasma IL-1β levels were significantly higher and plasma IL-1Ra levels were significantly lower in our DN patient cohort than in HCs. These results reinforced earlier work by Niknami et al. [25] showing that DN patients had significantly higher levels of IL-1β in comparison to diabetic patients without nephropathy. There is accumulating evidence that the inflammasome and IL-1 cytokines are central elements in the pathogenesis of diabetic kidney disease [26, 27]. In particular, IL-1β triggers the activation of all types of leukocytes and renal cells, inducing tubulointerstitial fibrosis [28]. Furthermore, in both the total of 109 individuals and in the DN group, the IL-1β levels were higher in subjects homozygous for the T allele variant of IL-1β (−511C/T) compared with heterozygous individuals. This is consistent with the previous findings by Ioannis et al. [29] showing that a higher plasma IL-1β level was associated with the TT genotype. However, the CC allele was found to be associated with a higher IL-1β mRNA level through a dominant model [30]. In the present study, IL-1β plasma levels were significantly higher for the CC allele than for the TC allele in diabetic patients, but due to the relatively small sample size of patients carrying the CC allele in our diabetic cohort, we were unable to demonstrate an association between the CC allele and the plasma IL-1β levels for the TC and TT alleles.

To our knowledge, there have been few studies regarding the relations between IL-1β and IL-1Ra SNPs and DN. Our results strengthen the evidence for a role of the IL-1β (−511C/T) polymorphism in DN.

However, this study has several limitations. First, the sample size was limited. Additional studies with larger sample sizes and more detailed studies of the T and C alleles are required to elucidate the impact of the IL-1β (−511C/T) gene polymorphism in DN. Second, this study was carried out in a population from northern Guangxi. The genetic association between IL-1β (−511C/T) gene polymorphisms and IL-1β plasma levels in DN patients may be different in populations of different ethnicities. Third, we cannot exclude the effects of other confounding factors, including nutrition, lifestyle factors, smoking, and treatment factors. Finally, we did not include a group of T2DM patients without DN. Therefore, additional larger studies also including diabetic patients without DN are required to examine whether the changes described above are due to T2DM per se or are specific for diabetic patients with DN.

5. Conclusions

In conclusion, the present study suggests that the IL-1β (−511C/T) polymorphism is significantly correlated with increased DN susceptibility in the population of northern Guangxi, with the T allele as a risk factor, which may contribute to the pathogenesis of DN. Further study is required to validate the clinical significance of these findings.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Xueling Liao and Yanan Xiao contributed equally to this work.

Acknowledgments

This work was supported by the National Natural Science Foundation of China, Grant/Award Number: 81360117; Guangxi Medical and Health Appropriate Technology Development and Application Project, Grant/Award Number: S2017010; Young and Middle-Aged Faculty Research Ability Enhancement Project, Grant/Award Numbers: 2018glmcy036 and 2018glmcy077.

Supplementary Materials

Figure 1: IL-1β (−511C/T) genotyping plot. Figure 2: IL-1β (+3953C/T) genotyping plot. Figure 3: IL-1Ra (+8006C/T) genotyping plot. (Supplementary Materials)

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