Research Article

Association between interleukin-10 gene rs1800896 polymorphism and diabetic retinopathy in a Chinese Han population

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Introduction

Diabetic retinopathy (DR), the most common single cause of newly reported cases of blindness amongst adults [1], is a neurovascular complication of type 2 diabetes mellitus (T2DM) [2–4]. DR is characterized by injuries in neural and vascular structures [3] and is increasingly reported to be greatly influenced by inflammation [5]. The levels of cytokines, inflammatory cells, and angiogenic factors reportedly increase following DR [6]. The development and progression of DR may involve interleukin-10 (IL-10), an anti-inflammatory cytokine with potent deactivating properties.

IL-10 is expressed by most cells of the adaptive and innate immune systems, including dendritic cells, leukocytes, and macrophages [7]. IL-10 gene rs1800896 polymorphism (IL-10 -1082G/A polymorphism) in the promoter region could affect the IL-10 expression [8]. The IL-10 gene polymorphism is reportedly associated with the risk of DR in different populations [9–12]. However, this single nucleotide polymorphism (SNP) has not been investigated in Chinese populations. Therefore, we decided to investigate whether the IL-10-1082G/A polymorphism was associated with proliferative DR (PDR) in a Chinese Han population with T2DM.

Methods

Study population

In this hospital-based case–control design, totally 327 hospitalized T2DM patients with PDR and 461 T2DM patients without PDR were selected from the First Affiliated Hospital of Anhui Medical University between March 2014 and July 2017. T2DM was diagnosed according to the American Diabetes Association guidelines [13], while PDR was diagnosed through direct funduscopy examination. The stage of PDR was determined according to the retinopathy severity scale of the Early Treatment Diabetic Retinopathy Study.
Table 1 Clinical manifestations of PDRy in patients with type 2 diabetes

| Variable                        | With PDR (n=325) | Without DR (n=460) | P     |
|---------------------------------|------------------|--------------------|-------|
| Age (years)                     | 53.58 ± 12.81    | 52.89 ± 12.29      | 0.440 |
| Sex                             |                  |                    |       |
| Male                            | 165 (50.5%)      | 249 (54.1%)        |       |
| Female                          | 162 (49.5%)      | 212 (45.9%)        |       |
| BMI (kg/m²)                     | 34.98 ± 4.78     | 29.44 ± 5.92       | <0.001|
| LDL (mmol/l)                    | 3.19 ± 0.95      | 3.14 ± 0.97        | 0.534 |
| HDL (mmol/l)                    | 1.22 ± 0.28      | 1.20 ± 0.31        | 0.340 |
| TC (mmol/l)                     | 5.48 ± 1.57      | 5.36 ± 1.43        | 0.286 |
| Hypertension                    |                  |                    |       |
| Yes                             | 307 (93.9%)      | 400 (86.8%)        |       |
| No                              | 20 (6.1%)        | 61 (13.2%)         |       |
| Dyslipidemia                    |                  |                    |       |
| Yes                             | 301 (92.0%)      | 404 (87.6%)        | 0.047 |
| No                              | 26 (8.0%)        | 57 (12.4%)         |       |
| Duration of diabetes (years)    | 15.52 ± 5.86     | 16.30 ± 7.06       | 0.003 |

Study Research Group. The inclusion criteria were (i) no need of permanent insulin treatment within the first year of diagnosis, (ii) no previous episodes of ketoacidosis, and (iii) age at diagnosis of diabetes ≥ 30 years. People with coronary artery diseases, peripheral vascular diseases, history of any thrombotic event, acute infection, or any other ocular disorders (e.g. branch retinal venous occlusion, glaucoma, Eales disease) were excluded. The demographic, lifestyle and clinical characteristics of all subjects were collected from medical records, including age, gender, body mass index (BMI); kg/m²), hypertension and dyslipidemia. This case–control study was approved by the Ethics Committee of the Hospital and performed according to Declaration of Helsinki. All patients provided written informed consent prior to participation.

Genomic DNA extraction and genotyping

Blood samples were collected using vacutainer tubes and then transferred to ethylene- diamine-tetra-acetic acid (EDTA) tubes. DNA was extracted using a Biopur Mini Spin kit (Biometrix). The genotypes of IL-10 gene polymorphism were identified by PCR-based restriction fragment length polymorphism (RFLP) assay. The PCR program was as follows: initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s; final extension at 72°C for 10 min. To ensure the genotyping quality, two independent investigators interpreted the images of each gel, and at least 10% of the samples were randomly selected for repeated genotyping.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) of the SNP genotypes was accessed by the goodness-of-fit Chi-square (χ²) test to compare the observed and expected genotype frequencies amongst controls. Associations between demographic characteristics and IL-10 gene rs1800896 polymorphism genotypes were assessed through χ² test (for categorical variables) and Student’s t test (for continuous variables). Associations between the IL-10 gene rs1800896 polymorphism A/G genotypes and the risk of PDR were estimated by calculating odds ratios (ORs) and 95% confidence intervals (CIs) using logistic regression analysis. All statistical analyses were performed on SAS software package 9.1.3 (SAS Institute, Cary, NC, U.S.A.) with the significance level at P < 0.05.

Results

Clinical information of the study population

The characteristics of the subjects are summarized in Table 1. The cases and controls were aged 53.58 and 52.89 years on an average, respectively, and involved 50.5 and 54.1% of males, respectively, indicating the two groups were well matched in terms of age and gender (both P > 0.05). TC, HDL, and LDL values are listed in the left column. Significant association was found in the analyses of hypertension and dyslipidemia between cases and controls, indicating hypertension and dyslipidemia are important factors for the DR development in the Chinese Han population.
### Table 2 Logistic regression analysis of associations between IL-10 rs1800896 polymorphism and risk of PDR

| Genotype | With PDR* (n=325) | Without DR* (n=460) | OR (95% CI) | P |
|----------|-------------------|---------------------|-------------|---|
|          | n                 | %                   | n           | %       |               |             |
| AA       | 124               | 31.7                | 146         | 31.7    | 1.00         |              |
| AG       | 153               | 48.7                | 224         | 48.7    | 0.80; (0.59–1.10) | 0.176 |
| GG       | 48                | 19.6                | 90          | 19.6    | 0.63; (0.41–0.96) | 0.031 |
| AG+GG    | 201               | 63.8                | 314         | 68.3    | 0.75; (0.56–1.02) | 0.063 |
| AG+AA    | 277               | 85.3                | 370         | 80.4    | 1.00         |              |
| GG       | 48                | 19.6                | 90          | 19.6    | 0.71; (0.49–1.05) | 0.083 |
| A        | 401               | 56.1                | 516         | 56.1    | 1.00         |              |
| G        | 249               | 43.9                | 404         | 43.9    | 0.79 (0.65, 0.97) | 0.027 |

*The genotyping was successful in 325 cases and 460 controls. Bold values are statistically significant (P<0.05).

### Table 3 Stratified analyses between IL-10 rs1800896 polymorphisms and PDR

| Variable          | rs1800896 (with PDR/without DR) | AG vs. AA | GG vs. AA | GG+AG vs. AA | GG vs. AG+AA |
|-------------------|---------------------------------|-----------|-----------|--------------|--------------|
| Sex               |                                 | AG        | GG        | GG+AG        | GG           |
| Male              | 63/82                           | 0.85 (0.54–1.31); 0.453 | 0.65 (0.37–1.16); 0.142 | 0.78 (0.52–1.18); 0.247 | 0.72 (0.43–1.20); 0.206 |
| Female            | 61/64                           | 0.75 (0.48–1.19); 0.222 | 0.61 (0.32–1.14); 0.122 | 0.72 (0.47–1.10); 0.150 | 0.72 (0.41–1.27); 0.258 |
| Hypertension      |                                 |           |           |              |              |
| Yes               | 113/121                         | 0.79 (0.56–1.10); 0.159 | 0.62 (0.40–0.97); 0.085 | 0.74 (0.54–1.01); 0.058 | 0.71 (0.48–1.06); 0.098 |
| No                | 11/25                           | 0.57 (0.18–1.78); 0.352 | 0.57 (0.13–2.42); 0.445 | 0.57 (0.21–1.57); 0.276 | 0.72 (0.18–2.87); 0.642 |
| Dyslipidemia      |                                 |           |           |              |              |
| Yes               | 112/128                         | 0.83 (0.59–1.16); 0.267 | 0.65 (0.42–1.02); 0.059 | 0.78 (0.57–1.06); 0.116 | 0.73 (0.49–1.09); 0.119 |
| No                | 12/18                           | 0.59 (0.22–1.62); 0.305 | 0.41 (0.09–1.78); 0.234 | 0.54 (0.21–1.40); 0.203 | 0.55 (0.14–2.15); 0.386 |
| Age (years)       |                                 |           |           |              |              |
| <55               | 61/84                           | 0.97 (0.63–1.49); 0.892 | 0.69 (0.39–1.22); 0.198 | 0.88 (0.59–1.33); 0.552 | 0.70 (0.42–1.17); 0.170 |
| ≥55               | 63/62                           | 0.65 (0.41–1.03); 0.067 | 0.57 (0.30–1.09); 0.090 | 0.63 (0.40–0.98); 0.038 | 0.74 (0.41–1.32); 0.305 |
| BMI               |                                 |           |           |              |              |
| <25               | 3/29                            | 0.54 (0.10, 2.83); 0.486 | 0.35 (0.03, 3.52); 0.389 | 0.47 (0.10, 2.23); 0.344 | 0.49 (0.06, 4.28); 0.522 |
| ≥25               | 121/117                         | 0.85 (0.61, 1.19); 0.354 | 0.73 (0.46, 1.16); 0.182 | 0.82 (0.60, 1.13); 0.223 | 0.80 (0.55, 1.21); 0.298 |

*The genotyping was successful in 325 cases and 460 controls. Bold values are statistically significant (P<0.05).

### Association between IL-10 gene rs1800896 polymorphism and PDR risk

The genotype distributions of IL-10 gene rs1800896 polymorphism in the controls conformed to the HWE (Table 2). Logistic regression analyses revealed GG genotype or G allele was related to decrease the risk for PDR (Table 2). We also evaluated the effects of the SNP on PDR risk according to patient characteristics (Table 3). The association between the rs1800896 polymorphism and PDR was only observed amongst the hypertensive subjects and older subjects (≥55 years), but was independent of gender or dyslipidemia. No significant association was found between genotype and the clinical or biochemical characteristics (Table 4).
Table 4 The clinical and biochemical characteristics of rs1800960 polymorphism amongst two groups

|                | With PDR (n=325) | Without DR (n=460) |
|----------------|-----------------|-------------------|
|                | AA (n=124)      | AG (n=153)        | GG (n=48) | P     | AA (n=146) | AG (n=224) | GG (n=90) | P     |
| Age (years)    | 55.14 ± 13.34   | 52.91 ± 12.10     | 51.81 ± 13.65 | 0.207 | 52.37 ± 12.17 | 53.19 ± 12.45 | 52.87 ± 12.21 | 0.823 |
| BMI (kg/m²)    | 34.65 ± 4.77    | 35.03 ± 4.78      | 35.78 ± 4.86  | 0.381 | 30.24 ± 6.24  | 29.16 ± 5.60  | 28.70 ± 5.93  | 0.191 |
| TC (mmol/l)    | 5.57 ± 1.72     | 5.51 ± 1.48       | 5.21 ± 1.45   | 0.400 | 5.27 ± 1.42   | 5.44 ± 1.43   | 5.32 ± 1.45   | 0.525 |
| HDL (mmol/l)   | 1.22 ± 0.27     | 1.21 ± 0.28       | 1.23 ± 0.31   | 0.961 | 1.20 ± 0.29   | 1.21 ± 0.31   | 1.19 ± 0.32   | 0.935 |
| LDL (mmol/l)   | 3.19 ± 0.92     | 3.21 ± 1.00       | 3.06 ± 0.88   | 0.643 | 3.13 ± 0.97   | 3.12 ± 0.97   | 3.24 ± 1.01   | 0.611 |

Discussion

This case–control study showed that IL-10 gene rs1800896 polymorphism was related to decreased risk for PDR in a Chinese population.

DR is proposed to be a manifestation of a persistent low-grade inflammation [14]. IL-10 can inhibit the production of pro-inflammatory cytokines and stimulate differentiation, proliferation and survival of some immune cells [7]. The IL-10 deficiency may not elicit protective immune response, but excessive production can exaggerate inflammatory response, resulting in immunopathology and tissue damage.

Recently, several studies explored the association between IL-10 gene rs1800896 polymorphism and DR risk. A Caucasian study first uncovered an association between this SNP and the PDR development in T2DM and demonstrated that the GG genotype of this polymorphism was associated with increased risk of PDR [12]. Later, a study from India investigating the relationship between this SNP and PDR risk found GG genotype or G allele of this polymorphism was associated with increased risk for PDR [10]. Two Brazilian studies yielded conflicting findings, as Rodrigues et al. [11] did not obtain an association, while da Silva Pereira et al. [9] found rs1800896 polymorphism increased the risk of non-PDR (NPDR), but not in PDR. Moreover, AA genotype of the rs1800896 polymorphism was independently associated with increased risk of NPDR, but the GG genotype was not associated with increased risk of PDR [9]. Thus, what reasons could explicate the conflicting findings? The first reason was clinical heterogeneity, since the study subjects were DR patients [11] or NPDR or PDR patients [9]. We assumed different types of DR showed genetic heterogeneity. Second, the discrepancy may be explained by different living environments and lifestyles, although they were both Brazilians. Third, the limited sample sizes [11] might not have sufficient power to reach a convincing conclusion compared with other studies.

The present study showed GG genotype or G allele carriers were related to decreased risk for PDR. Different genetic backgrounds, living environments, sample sizes, exposure factors, and clinical phenotypes of PDR may account for conflicting results of the above studies. The stratified analyses showed the risk of PDR conferred by the IL-10 gene rs1800896 polymorphism remained significant in the hypertensive and older subgroups (age ≥ 55 years), which was because susceptible individuals are likely to expose to risk factors. However, the results should be interpreted with caution because of the limited sample sizes in the stratified analyses and the limited power. Additionally, no significant association between genotype and the clinical or biochemical characteristics was observed in the present study. Nevertheless, our findings still provide evidence for a possible interaction between the rs1800896 polymorphism and some PDR risk factors.

Potential limitations of the present study should be considered. First, we only investigated PDR, but not NPDR. Second, our results were based on unadjusted estimates for confounding factors. Third, the sample size was not large enough, which might underpower our work. Fourth, no details about DR severity or treatment response were obtained, which restricted our analyses. Fifth, the underlying mechanisms of this SNP in DR should also be investigated. Sixth, multiple comparisons included in the analyses (five genetic models, and stratification of four demographic and clinical factors) may result in some false positive associations. Finally, true significance of the association between this SNP and PDR risk should be supported by further studies in different populations.

In conclusion, this case–control study indicates that IL-10 gene rs1800896 polymorphism is associated with a decreased risk of PDR. Nevertheless, this finding should be verified by further multicenter well-designed studies with larger sample sizes that include gene–environment interaction assessment.

Author contribution

L.L. and J.Z. designed and performed the experiments. Y.X., J.G., L.F., and D.X. analyzed the data. L.L. and J.Z. wrote the present paper. J.G. and L.L. revised the manuscript.
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Competing interests
The authors declare that there are no competing interests associated with the manuscript.

Abbreviations
DR, diabetic retinopathy; HDL, High-density lipoprotein; HWE, Hardy–Weinberg equilibrium; IL-10, interleukin-10; LDL, Low-density lipoprotein; PDR, proliferative DR; NPDR, non-PDR; SNP, single nucleotide polymorphism; TC, total cholesterol; T2DM, type 2 diabetes mellitus.

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