

Original Article

***TNF- α -308G/A* polymorphism associated with *TNF- α* protein expression in patients with diabetic nephropathy**

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Abstract: Aims: This study was to clarify the regulated effects of *TNF- α -308G/A* polymorphism on *TNF- α* and investigate the relationship of *-308G/A* polymorphisms with diabetic nephropathy (DN) susceptibility. Methods: 86 DN patients and 94 healthy individuals were enrolled in our study. Polymerase chain reaction-sequence specific primer (PCR-SSP) detection technology was used to testify single nucleotide polymorphism (SNP) of *TNF- α* gene. Enzyme-linked immunosorbent assay (ELISA) was used to measure the content of *TNF- α* protein. Odds ratio (OR) with 95% CI were used to evaluate the association of *TNF- α -308G/A* polymorphism and DN susceptibility. Results: The level of *TNF- α* protein was much higher in DN patients compared to that of controls ($P < 0.05$). For *TNF- α -308G/A*, G/A genotype could increase the risk for DN (OR = 2.15, 95% CI = 1.08-4.30). Moreover, a allele frequency was found higher in cases compared with controls, which suggested that A allele served as an genetic-susceptibility factor for DN (OR = 1.89, 95% CI = 1.10-3.26). Further analysis indicated that level of *TNF- α* for individuals with mutant genotype (GA and AA) were higher than that of individuals with wild genotype ($P < 0.05$). However, AA genotype showed no effects on DN susceptibility (OR = 2.08, 95% CI = 0.56-7.33). Conclusion: *TNF- α -308G/A* polymorphism was associated with expression level of *TNF- α* and served as an genetic-susceptibility factor for DN.

Keywords: Tumor necrosis factor- α , diabetic nephropathy, protein expression

Introduction

Tumor necrosis factor- α (*TNF- α*) is a multifunctional pro-inflammatory cytokine, which is associated with some pathological processes, such as apoptosis, proliferation, inflammation and immunoregulation. Effects of single nucleotide polymorphisms (SNPs) within tumor necrosis factor- α (*TNF- α*) on inflammatory diseases have attracted extensive attention. Furthermore, some studies have confirmed that SNPs within *TNF- α* promoter affected the expression of *TNF- α* , which were related to the occurrence and severity of some inflammatory diseases [1-5], however, some studies have not come to any conclusion or even come to opposite conclusion [6-8].

It was generally accepted that *TNF- α -308G/A* polymorphism might affect the transcription of *TNF- α* , of which A allele could strengthen the transcription of *TNF- α* and G allele is associated with low-level expression of *TNF- α* [9, 10]. In addition, Navarro et al. found that expression

level of *TNF- α* is upregulated in patients of diabetic nephropathy [11]. Nevertheless, there was no report about association of *TNF- α -308G/A* polymorphism and diabetic nephropathy (DN) based on Chinese population.

In the study, we analyzed the association of *TNF- α -308G/A* polymorphism and the expression level of *TNF- α* protein, and further explored the role of *-308G/A* loci in the pathogenesis of DN.

Materials and methods

Subjects

According to the diagnosis and classification criteria for diabetes mellitus of World Health Organization (WHO) in 1999, 86 patients with DN were enrolled. The control group was 94 healthy checkers in the hospital, who were required for no histories of high blood pressure and coronary heart disease. The subjects were unrelated Chinese, and the controls were matched to cases in age and gender.

Table 1. Primers sequence of TNF- α -308

Primers	Sequence
Normal primer	F: 5'-GATGGACTCACCAGGTGAG-3' R: 5'-CTCATGGTGTCTTTCCAGG-3'
Primer of -308A	5'-GTGGAGGCTGAPLCCCCGTCCT-3'
Primer of -308G	5'-GTGGAGGCTGAPLCCCCGTCCT-3'

Methods

DNA extraction: 5 ml peripheral blood was obtained from each subject, and then processed with anticoagulation by 5% EDTA. Genome DNA was extracted from blood with the method of Resse et al [12].

PCR amplification: Primers were provided by American Lambda Biotec, co, LTD. Primer sequence was shown in **Table 1**. PCR reaction was performed under the following conditions: pre-denaturation 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 60°C for 50 s, 72°C for 40 s and final 72°C for 10 min. PCR products were detected with 2% agarose electrophoresis for 15-20 min.

ELISA assay: Serum TNF- α level of cases and controls were determined with ELISA kit (Beijing Tiantan Biological Products co., LTD).

Statistical treatment: Hardy Weinberg Equilibrium (HWE) was tested by χ^2 test. T-test was adopted to calculate the comparison in TNF- α protein level between two groups or mutant and wild genotypes. χ^2 test was used to evaluate the differences in genotype distribution between cases and controls. Odds ratio (OR) with 95% confidence interval (CI) were used to assess the relationship of TNF- α -308 and DN susceptibility. All the analysis were conducted in SPSS 18.0. If $P < 0.05$, there is statistical significance.

Results

Comparisons of genotype and allele frequencies of TNF- α -308 in two groups

Genotype frequencies of TNF- α -308 in case and control groups were consistent with HWE ($P > 0.05$), which suggested the population in the study was representative. As shown in **Table 2**, frequency of G/A genotype was found higher in case group than that of controls (32.6% vs. 19.1%), which indicated that G/A

genotype could increase the risk for DN (OR = 2.15, 95% CI = 1.08-4.30). However, A/A genotype showed no significant association with DN risk (OR = 2.08, 95% CI = 0.56-7.33). Additionally, risk for DN of A allele carriers was 0.89 times than that of G allele (OR = 1.89, 95% CI = 1.10-3.26).

Expression level of TNF- α

In the study, we measured the content of serum TNF- α in the case and control groups.

The result indicated that expression level of TNF- α was remarkably upregulated in DN patients ($P < 0.05$) (**Figure 1**).

Association of TNF- α -308 polymorphism and TNF- α level

And then we investigated the association of -308 polymorphism and expression level of TNF- α . As shown in **Figure 2**, expression level of TNF- α for patients with mutant genotype (GA and AA) was much higher than that of patients with mutant genotype ($P < 0.05$).

Discussion

DN, the most common chronic microvascular complication of diabetes mellitus (DM), seriously affects living quality of the patients. Inflammation, cell hypertrophy and dedifferentiation contribute to the progression of DN [13]. The occurrence of DN is related to various factors including oxidative stress, high glucose, hemodynamics change, metabolic disorder and genetic factor [14, 15]. And the role of genetic factor in the pathogenesis of DN attracted much more attention.

TNF- α gene located at 6p21.3, is a key member in the central part of major histocompatibility complex (MHC) [16]. And TNF- α is an important inflammatory mediator, produced by T cell and macrophages [17]. Recently, the studies have found that TNF- α is also produced by multiple cells in kidney, such as glomerular mesangial cell (GMC), proximal tubular cell and blood cell in kidney [18], among which GMC plays a crucial role in the progression of DN and the function change of GMC increases the risk of DN [19].

The study conducted by Baudl et al. with the method of Northern blot suggested that TNF- α

Table 2. Genotype and allele frequencies of *TNF-α*-308 in case and control groups

Genotype/Allele	DN cases n (%)	Controls n (%)	OR (95% CI)	χ^2	P value
Genotype					
-308G/A					
G/G	52 (60.5)	72 (76.6)	1.00	-	-
G/A	28 (32.6)	18 (19.1)	2.154 (1.079-4.300)	4.828	0.038
A/A	6 (6.9)	4 (4.3)	2.077 (0.558-7.332)	1.230	0.328
Allele					
G	132 (76.7)	162 (86.2)	1.00	-	-
A	40 (23.3)	26 (13.8)	1.888 (1.095-3.255)	5.330	0.029

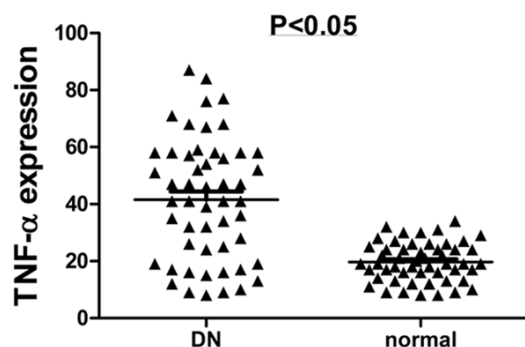


Figure 1. Comparison of expression level of *TNF-α* in two groups.

mRNA expression level was low in the unactivated GMC [20], while it was upregulated with lipopolysaccharide (LPS) activation [21]. For GMC, not only endotoxin, but also fragment of complement, immune complex, platelet-derived growth factor, epidermal growth factor, platelet activating factor and interleukin could trigger the expression of *TNF-α* [22]. In addition, *TNF-α* content is found to associated with pathological changes of mesangial proliferative glomerulonephritis (MsPGN) [23-25], which indicated that *TNF-α* might one of important factor resulted in proliferation, sclerosis, disease progression of GMC.

In consideration of the role of *TNF-α* for GMC, the study focusing on the association of *TNF-α* and DN is valuable. In the study, we took -308 polymorphism into consideration, which is related to the expression level of *TNF-α*. And the result found that the expression level of *TNF-α* is much higher in DN patients than that of controls. Further analysis suggested that *TNF-α* protein level of individuals with mutant genotype was higher than that of individuals with wild genotype. Moreover, G/A genotype could increase the risk for DN. So we concluded

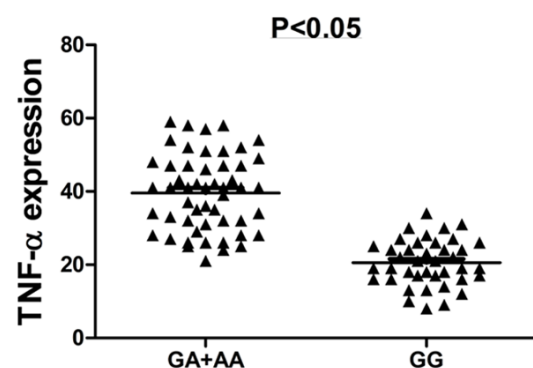


Figure 2. Comparison of expression level *TNF-α* between mutant and wild genotype.

that *TNF-α* -308 polymorphism was a genetic-susceptibility factor for DN, which would contribute to the prevention and early diagnosis of DN.

Disclosure of conflict of interest

None.

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