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CXCL5 Gene Polymorphism Association with Diabetes Mellitus

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Abstract

Background: CXCL5, also known as epithelial cell-derived neutrophil-activating peptide (ENA-78), is a chemokine that has a role in the development of cardiovascular and other diseases. We have previously scanned the full length *CXCL5* gene and reported the −156G>C (rs352046) polymorphism in the promoter region of this gene.

Objective: The aim of this study was to examine whether there was an association between this polymorphism and type 2 diabetes mellitus or its microvascular complications in an Iranian population.

Methods: A total of 230 patients with type 2 diabetes were recruited from Rafsanjan, in the south-east of Iran; 102 healthy control subjects were recruited from the same area. The region containing the *CXCL5* −156G>C polymorphism was genotyped by PCR amplification and restriction fragment length polymorphism analysis, and allele frequency data were analyzed using STATA 8 software.

Results: We observed that patients with type 2 diabetes had a higher frequency of carrying either the G/C or C/C genotype compared with healthy controls (C/G + C/C vs G/G; p = 0.004; odds ratio [OR] 2.17; 95% CI 1.27, 3.80). In addition, the frequency of allele C was significantly increased in patients with diabetes compared with controls (p = 0.01; OR 1.72; 95% CI 1.07, 2.86). No association was found between this polymorphism and diabetic microvascular complications.

Conclusions: Our findings suggest a role of CXCL5 in the pathogenesis of diabetes. The mechanism behind this role needs to be investigated further. Moreover, replications in other populations with larger sample sizes are required to confirm these findings.

Background and Objective

CXCL5, also known as epithelial cell-derived neutrophil-activating peptide (ENA-78), belongs to the CXC subfamily of chemokines and is expressed by epithelial cells after stimulation with pro-inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor-α (TNFα), inducing polymorphonuclear neutrophil (PMN) adhesiveness.[1] Zimmerman and colleagues[2] have shown that CXCL5 is also released by stimulated endothelial cells in human lung and other tissues, and can act in concert with IL-8 to induce neutrophil pro-adhesive activity. Higher levels of CXCL5 have been detected in patients with chronic pancreatitis compared with healthy individuals; and levels are also elevated in patients with severe acute pancreatitis, suggesting the involvement of this chemokine in the initiation and perpetuation of disease.[3,4] It has also been observed as being an important chemokine expressed in a number of inflammatory diseases such as Crohn disease, ulcerative colitis, and rheumatoid arthritis.[5,6] The production of CXCL5 from endothelial cells has been shown to be

reduced after treatment with atrovastatin, which suggests that the chemokine has a possible role in cardiovascular drug response.[7]

The *CXCL5* gene has been mapped to chromosome 4q13-q21 (in the same region as other CXC subfamily genes) and consists of four exons and three introns, resembling the organization of the *IL8* gene.[8] We have previously scanned the full sequence of the gene and reported two single nucleotide polymorphisms (SNPs): the promoter polymorphism −156G>C (rs352046) and a synonymous 398G>A (rs425535) polymorphism in exon 2.[9] The allele and genotype frequencies for these polymorphisms were reported to be high in European and US populations;[9-11] furthermore, a functional role has been described for the −156G>C polymorphisms and it was shown that carriers of the variant C allele have higher circulating and leukocyte-elaborated CXCL5 concentrations.[12]

The aim of this study was to examine the association between this functional *CXCL5* −156G>C (rs352046) promoter polymorphism and type 2 diabetes mellitus or diabetes microvascular complications compared with healthy control subjects in an Iranian population.

Research Design and Methods

Subjects Characteristics

The study group comprised 230 patients with type 2 diabetes who were recruited from the diabetes clinic at the Aliebn Abitaleb Hospital, Rafsanjan University of Medical Sciences, in Rafsanjan, in the south-east of Iran. The control group consisted of 102 healthy individuals recruited from the same area.

All the subjects selected for this study were of Fars ethnic origin. Population admixture is rare in this region and subjects with other ethnic backgrounds were not entered in this study.

After registration and providing personal and demographic data, 3–5 mL of venous bloods were collected in EDTA tubes from each participant and stored at −20°C for DNA extraction. The study was approved by the ethics committee of Tehran University. Informed consents were obtained from all the patients enrolled in the study.

Diagnostic Criteria

Patients with diabetes were diagnosed according to the American Diabetes Association criteria.[13] Diabetic retinopathy was diagnosed by an expert ophthalmologist and was based on ophthalmoscopic examination. Diabetic nephropathy was defined as microalbuminuria of more than 30 mg/24 h in two to three samples with exclusion of other conditions that can cause proteinuria. Neuropathy was defined by symptoms or signs according to the Diabetes Control and Complication Trial criteria.[12] Patients with neuropathic foot ulcers were defined as neuropathy.

DNA Extraction and Genotyping

DNA was extracted from anticoagulated blood collected in EDTA tubes using the salting-out method.

Genotyping was performed using PCR-restriction fragment length polymorphism (RFLP) assay. The following primers were used:

- forward: 5′-CTCCTCCTGGCCACCCTCGC-3′;
- reverse: 5′-TCAAGCTTTGGGATGCTGGGGGA-3′.

The PCR cycles were as follows: 95°C for 15 minutes followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The final extension was carried at 72°C for 7 minutes. The presence of PCR product (114 basepairs) was verified on a 2% agarose gel stained with ethidium bromide. PCR products were digested with NruI restriction enzyme. The digest was incubated overnight at 37°C. NruI restriction endonuclease digested the PCR product yielding DNA fragments of 19 and 95 basepairs when G allele was present. The products of the digest were then visualized on a 3.5% agarose gel stained with ethidium bromide.

Our genotyping method was validated using samples with known genotypes from Spanish and US populations and results were replicable by using either Pyrosequencing® (Biotage, Uppsala, Sweden) or Taqman® (TaqMan® Gene Expression Assays; Applied Biosystems, Foster City, CA, USA) systems.

Table I. *CXCL5* −156G>C polymorphism allele and genotype frequencies in patients with type 2 diabetes mellitus and controls

Parameter	Diabetes	Controls
No. of patients	230	102
Genotype[a]		
G/G	141 (61%)	79 (77%)
G/C	82 (36%)	19 (19%)
C/C	7 (3%)	4 (4%)
Allele (2N)[b]		
G	364 (79%)	177 (87%)
C	96 (21%)	27 (13%)

a G/C + C/C vs G/G; p = 0.004; OR 2.17; 95% CI 1.27, 3.80.

b C allele vs G allele; p = 0.01; OR 1.72; 95% CI 1.07, 2.86.

OR = odds ratio.

Table II. Genotype frequency of *CXCL5* −156G>C polymorphism in diabetic patients with and without neuropathy, nephropathy, and retinopathy

Genotype	G/G	G/C	C/C	p-Value
Neuropathy	93 (64%)	48 (33%)	5 (3%)	0.5
No neuropathy	46 (58%)	32 (40%)	2 (2%)	
Nephropathy	11 (55%)	9 (45%)	0 (0%)	0.4
No nephropathy	123 (63%)	67 (34%)	6 (3%)	
Retinopathy	26 (66%)	12 (31%)	1 (3%)	0.8
No retinopathy	108 (62%)	62 (35%)	5 (3%)	

Statistical Analysis

The strength of association between different groups and alleles or genotypes of the *CXCL5* gene polymorphism was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either Chi-square or Fisher exact analysis. All analyses were carried out using the STATA 8 (StataCorp LP, College Station, TX, USA) software.

Results

The male/female ratio was 69/161 in diabetic patients and 62/40 in controls. The mean age was 53 ± 10 in diabetic patients and 50 ± 10 in healthy controls. The mean body mass index (BMI) was 27 ± 4 in patients with diabetes and 23 ± 3 in healthy controls.

CXCL5 Allele and Genotype Frequencies in Patients with Type 2 Diabetes Compared with Healthy Controls

The allele and genotype frequencies of the *CXCL5* −156G>C polymorphism conformed to Hardy-Weinberg equilibrium in both patients and the healthy control populations. When we compared the frequency of the *CXCL5* gene polymorphism in the patients with diabetes and healthy controls, we observed that the frequency of G/C or C/C genotype in patients with diabetes was significantly higher compared with the controls (C/G + C/C vs G/G; p = 0.004; OR 2.17; 95% CI 1.27, 3.80) [table I]. This was still significant when we adjusted for age, BMI, and sex using logistic regression analysis (OR 1.98; 95% CI 1.08, 3.62). In addition, the frequency of the C allele was significantly increased in patients with diabetes compared with healthy controls (p = 0.01; OR 1.72; 95% CI 1.07, 2.86) [table I].

CXCL5 Allele and Genotype Frequencies in Patients with and without Diabetic Complications

There was no significant difference in the allele or genotype frequencies of the *CXCL5* −156G>C polymorphism in patients with or without diabetic neuropathy or diabetic retinopathy (table II). However, the frequency of G/C genotype was increased in patients with nephropathy (45%), but this did not reach a significant level when compared with patients without nephropathy (table II).

Discussion

In this study we observed a significant association between the *CXCL5* promoter polymorphism –156C allele and type 2 diabetes in an Iranian population. This was not the case for the different subsets of diabetes, neuropathy, neuropathy, or retinopathy. However, the frequency of genotype G/C was relatively high in patients with diabetic nephropathy. This might be explained by the small sample size in our study. In a recent report by Zineh et al.[14] on the functional implication of this polymorphism, CXCL5 plasma concentrations were shown to be higher in individuals carrying a C allele. They also demonstrated that the C allele has an effect on leukocyte production of CXCL5.[14]

In previous studies no association was found between this polymorphism and primary vasculitis in a Spanish population.[11] Reports have described the frequency of this polymorphism in Caucasians within British, Spanish, and American populations, allele and genotypic frequencies were similar in all three populations.[9-11] The frequency of variant alleles was slightly lower in the healthy Iranian population (13% for the C allele versus 16% in other populations).

The association between the *CXCL5* gene polymorphism and diabetes found in this study suggests a role for the CXCL5 chemokine in the development or pathogenesis of diabetes. This role possibly involves neutrophil activation, though the mechanism of CXCL5 action in diabetes requires further investigation. Replication of these findings in other populations and with larger sample sizes is also required to confirm our data.

Acknowledgments

Dr Zineh and the University of Florida have filed a US patent application (#20080045582) for using *CXCL5* polymorphism and ENA-78 protein concentrations as diagnostic and prognostic tools. No ruling has yet been made.

The other authors declare no conflicts of interest.

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