ORIGINAL CONTRIBUTIONS

ASSOCIATION OF IL-4 AND IL-1RN (RECEPTOR ANTAGONIST) GENE VARIANTS AND THE RISK OF TYPE 2 DIABETES MELLITUS: A STUDY IN THE NORTH INDIAN POPULATION

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ABSTRACT

BACKGROUND: Inflammation is a key event closely associated with the pathophysiology of type 2 diabetes mellitus (T2DM). Association of genetic polymorphisms of inflammatory cytokines with T2DM is largely unknown. Our objective was to investigate the relationship of polymorphism of IL-1RN and IL-4, two important biomarkers of inflammation, with the risk of T2DM.

SETTING AND DESIGN: We recruited 120 clinically diagnosed T2DM patients and 150 normal healthy controls for this study in order to evaluate the nature of polymorphisms of IL-1RN and IL-4.

MATERIALS AND METHODS: Genomic DNA was isolated from the blood of all subjects, and the variable number of tandem repeat (VNTR) polymorphisms of IL-1RN and IL-4 genes was identified by polymerase chain reaction. Statistical analysis used: Genotype distribution and allelic frequencies were compared between patients and control group. Means, as well as odds ratios (ORs) with 95% confidence intervals (CI), were calculated using SPSS software (version 11.5). Results: Our study revealed that distribution of both IL-4 and IL-1RN (VNTR) gene polymorphisms were significantly associated with T2DM subjects. We, however, failed to find any association of gene-gene (IL-4 and IL-1RN) interaction with T2DM. Conclusions: Both IL-4 and IL-1RN (VNTR) gene polymorphisms were significantly associated with T2DM subjects. This may suggest that the genetic polymorphisms of IL-4 and IL-1RN genes could serve as susceptibility indicators for T2DM in the Indian population, but the actual mechanism of these associations will require more elaborate investigations. Lack of association of gene-gene (IL-4 and IL-1RN) interaction with T2DM may indicate the independent nature of influence of both these genes on the risk of T2DM.

Keywords: IL-4, IL-1RN, polymorphism, type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is manifested by hyperglycemia resulted from resistance to insulin in fat, muscle, and other key target tissues of insulin; and decreased insulin secretion by beta cells of islets of Langerhans. Chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of T2DM.[11] Recently, T2DM has also been recognized as an immune-mediated disease leading to impaired insulin signaling and selective destruction of insulin producing beta-cells in which cytokines play an important role.[3,4] Cross-sectional studies have provided support for the hypothesis that chronic subclinical inflammation may be associated with insulin resistance and may precede the development of clinically overt T2DM.[5]

IL-4 is an important anti-inflammatory cytokine that directs macrophages towards a phenotype that is characterized by the elaboration of other anti-inflammatory molecules, like IL-10, IL-1RN, and IL-1R2. IL-4 shifts the inflammatory balance by inhibiting the secretion of the pro-inflammatory cytokines IL-1beta, TNF-alpha, and IL-6 from macrophages. Disturbance of the anti-inflammatory response could be a critical component of the chronic inflammation found in T2DM. Type 2 diabetes mellitus disrupts anti-inflammatory cytokine function of IL-4 as a mediator of production of antagonist of interleukin-1 (IL-1RN).[6]

IL-1RN, a naturally occurring competitive inhibitor of IL-1, binds to the type I receptor and protects human pancreatic cells from IL-1beta–induced functional impairment and apoptosis. [7-8] Claus et al. proposed that IL-1RN has possible therapeutic potential in the treatment of T2DM. [8] Recently, it was indeed revealed that administration of IL-1RN in the form of Anakinra has been successfully shown to improve beta cell function in patients with T2DM.[9]

The nature of association of IL-4 and IL-1RN polymorphisms with, and their combined effect on, Indian T2DM patients is not known. As a first step to evaluate whether these cytokines’ polymorphisms have functional influence on the susceptibility to T2DM, we conducted a study of the association of the gene polymorphisms of IL-4 and IL-1RN with diabetes in a population-based study in the Indian population.

MATERIALS AND METHODS

Patients and clinical evaluation

The current study was carried out with prior approval from the institutional ethical committee. Patients with T2DM were enrolled from among the outpatients attending the diabetes clinic of a medical university from March to October 2007.

Screening and management of patients was done as per American Diabetes Association guidelines.[11] Subjects were included in the diabetes group if they had fasting glucose concentrations ≥126 mg/dL or 2-hour glucose concentrations ≥200 mg/dL after a 75-g oral glucose tolerance test with all clinical details.[12] A total of 120 subjects after screening were included in this study. A questionnaire was used to record clinical history of diabetes and associated complications, hypertension; as well as family history.

After screening with standard oral glucose tolerance test, a total of 150 age- and BMI-matched normal healthy controls were enrolled from among the healthy staff members of the institute and university for this study. Subjects having history of coronary artery disease or other metabolic disorders were excluded from the control group.
Sample processing and genotyping

After obtaining an informed consent from both the groups, 5 mL of blood sample was taken in Ethylenediamine tetraacetic acid (EDTA) and plain vials. Genomic DNA was extracted from peripheral blood leucocytes pellet using the standard salting-out method.[13]

IL-4 intron-3 VNTR

The region that contains the VNTR polymorphism of 70 bp within the IL-4 intron-3 was amplified by using the following PCR primer pairs: forward, 5'-TAGGCGAAGGGGAGGC-3'; and reverse, 5'-CTGTTACCTCACTGCTC-3'.[14]

The reaction was carried out in a total volume of 10 µL, containing genomic DNA (100 ng); 10 pmol of each primer, 1X Taq polymerase buffer and 0.5U of Taq DNA polymerase (Bangalore Genei, India). The cycling conditions were 95°C for 10 minutes, followed by 32 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 30 seconds, and final extension at 72°C for 8 minutes. Alleles of 183 bp (two repeats) and 253 bp were designated as B1 and B2 respectively and were analyzed by electrophoresis on a 2% agarose gel.[15]

The molecular weight of each band was determined by using the software in Biovis Gel Software, version 4 (Expert Vision, Mumbai), and the unknown samples were compared with the 100 bp DNA ladder (MBI-Fermentas, USA). To improve the genotyping quality and validation, 20% of samples were re-genotyped by other laboratory personnel, and the results were found to be reproducible with no discrepancy noticed in genotyping. Genotyping of 10% of samples was confirmed by DNA sequencing.

Statistical analysis

Allele frequencies, genotype frequencies, and carriage rates of the alleles in all the groups were compared with Fisher exact test using the program SPSS software (version 11.5, SPSS Inc., Chicago, IL). Data on quantitative characteristics were expressed as means ± SD. Comparisons between groups were made with the χ2 test (nominal data) or Student t test (interval data). Allele frequency was determined by using the software in Biovis Gel Software, version 4 (Expert Vision, Mumbai), and other parameters are listed in Table 1.

Two sided, and differences were considered statistically significant for P < 0.05. Hardy-Weinberg equilibrium was tested by the χ2 method. Odds ratio (OR) at 95% confidence interval (CI) was determined to describe the strength of association by logistic regression model. The sample size was calculated using the QUANTO ver. 1 program (http://hydra.use.edu/gxe). Calculated sample size was adequate to study both polymorphisms. Log additive inheritance model was used for T2DM as a polygenic disease.

RESULTS

A total of 120 T2DM patients (mean age, 48.41 ± 10.16 years) and 150 healthy controls (mean age, 50 ± 11.28 years) were evaluated for the IL-1RN and IL-4 gene polymorphism study. The mean blood pressure, plasma glucose, cholesterol (LDL/HDL/VLDL/total), serum creatinine, and other parameters are listed in Table 1.

Comparative analysis of combinations of IL-4 and IL-1RN (IL-4low–IL-1RNlow / IL-4high–IL-1RNhigh) showed that all these combinations lacked significant difference (P = 0.56, 0.99, and 0.84 respectively) among patients and controls. In regard to allele frequency and carriage rate, there was lack of association in case of IL-4 (P = 0.209 and .210 respectively) among patients and controls. However, a significant difference in both allele frequency and carriage rate (P < 0.01 and P = 0.041 respectively) was observed for IL-1RN gene polymorphism [Table 2]. The genotype frequency of BB (63.3% vs. 30.0%) and allele frequency of B (75.83% vs. 55.0%) of IL-1RN were significantly higher in patients than in controls. Similarly, in case of IL-4 the frequency of B2B2 genotype (65.0% vs. 50.67%) and that of B2 allele (77.08% vs. 72.33.0%) were significantly higher in patients than in controls [Table 2].

In order to investigate gene-gene interaction, we analyzed the combined effect of IL-4 and IL-1RN on, and their possible association with, T2DM. Genotypes were grouped into high- or low-producer phenotypes — IL-4, B1B1, or B1B2 = high-producer (HP) phenotypes; B2B2 = low-producer (LP) phenotypes. Similarly in case of IL-1RN, AA = HP phenotypes, and all others were considered LP phenotypes. Comparative analysis of combinations of IL-4 and IL-1RN (IL-4low–IL-1RNlow / IL-4high–IL-1RNhigh / IL-4low–IL-1RNhigh / IL-4low–IL-1RNhigh) showed that all these combinations lacked significant difference (P = 0.56, 0.99, and 0.84 respectively) between controls and T2DM patients [Table 2]. IL-4 and IL-1RN high-producing genotypes were taken as reference for this analysis.

We also analyzed the association of different clinical parameters of T2DM patients with IL-4 and IL-1RN genotypes; there was lack of association with any of these parameters [Table 3].
**DISCUSSION**

Earlier studies have reported that hyperglycemia associated with T2DM acutely increases peripheral cytokines like IL-6 and TNF-\(\alpha\) and IL-1 proteins.\(^{[16]}\) In vitro studies revealed that IL-6 and TNF-alpha can impair insulin-signaling pathway, resulting in insulin resistance.\(^{[17-18]}\)

It is proposed that cytokines might normally function in a feedback pathway to limit the number of adipocytes or lipid storage by exerting catabolic effects on the adipocyte, blocking lipid synthesis and lipoprotein lipase expression, while activating lipolysis, and can block or reverse differentiation of fibroblastic precursors into adipocytes.\(^{[19]}\) However, in vivo results till date are largely inconsistent. Identification of appropriate markers of T2DM for recognizing genetic influence upon initiation and progression of the disease might assist the clinicians in adopting a more precise approach for the identification of ‘high-risk’ T2DM patients and in the development of personal medicine strategies for targeting inflammatory components; thus meeting a crucial medical need, as well as enabling planning therapeutic interventions. Genetic polymorphisms studied so far in T2DM with TNF-\(\alpha\) and IL-6 have also revealed no or only marginal association.\(^{[20-21]}\)

Lisa et al. reported lack of association of IL-4 with type 1 diabetes mellitus, but the nature of association of IL-4 with T2DM is unknown.\(^{[22]}\) Recently, Achut et al. demonstrated a significant association of VNTR polymorphism of IL-4 with increased risk of T2DM, as well as its associated complications in the north Indian population.\(^{[23]}\) In our study, we also tried to find out the combined effect of IL-4RN and IL-4 on T2DM and observed that IL-4low–IL-1RNlow and IL-4high–IL1RNhigh may predispose to the development of T2DM in patients, although the \(P\) value was not significant.

Since both IL-1RN and IL-4 have been found to be associated with risk of T2DM, it was imperative to investigate if combination of their particular polymorphic variants could specifically influence the susceptibility further. Therefore, we also tried to find out the combined effect of IL-1RN and IL-4 on T2DM and observed that IL-4low–IL1RNlow genotype showed 1.26 times risk compared to IL-4low–IL-1RNhigh and IL-4high–IL1RNhigh. IL-1RNlow in patients, although the \(P\) value was not significant.

In conclusion, this study supports that genetic variation in IL-4 and IL-1RN cytokine genes may predispose to the development of T2DM in the Indian population. The combined effect of IL-4 and IL-1RN revealed a further increase in risk of T2DM.

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REFERENCES


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