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Molecular analysis of human population of Abbottabad Pakistan in relation to type-1 diabetes

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Abstract

Type 1 diabetes mellitus (T1DM) caused by the destruction of insulin producing beta cells of pancreas that are responsible for the autoimmune system of the individuals. Blood and urine glucose increases due to the lack of insulin in the body. The objective of the present study was to evaluate the role of IL-10 gene polymorphisms as susceptibility markers for Pakistani patients. IL-10 promoter polymorphisms (positions-592A/C) were analyzed in 183 individuals of Abbottabd. Ninety three known Pakistani patients with type-1 diabetes including 49 men and 44 women were recruited; having age ranged from 1.0 –70.0 years. Ninety one healthy individuals with no family history of diabetes were included as control. RFLP and PCR were used to detect allelic polymorphism of the IL-10 gene promoter region at position -592. Results indicated that the genotype frequency of the allel (-592A) was associated with type-1 diabetes. The genotype frequency of -592A allele was higher in adult patients than in old age patients. Our investigation suggests that IL-10 gene polymorphisms could be involved in the development of type-1 diabetes in the Pakistani population.

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Introduction

Type 1 diabetes mellitus (T1DM) is an important disease caused by the destruction of islet ß-cells (Atkinson and Maclaren 1994). In genetically susceptible individuals, it developed due to the destruction of ß-cells that depend on T-cells (Eisenbarth 2005). The major genes responsible for the susceptibility of the individual to type-1 diabetes lie within the major histocompatibility complex (MHC) region (Buzzetti *et al*, 1998). The insulin gene region (IDDM2) have been proposed as candidate genes (Lucassen *et al*, 1993). Among the major causes of pathogenesis of TIDM, the cytokines secreted by CD4 T helper type-1 and 2 (Th1 and Th2) cells may play important role (Almawi *et al*, 1999). Cytokines are of two types: Th1 (proinflammatory) cytokines such as TNF-a and interleukin-1, and Th2 (anti-inflammatory) cytokines such as interleukin-10 and interleukin-4 (Keen 2002). The exact roles, that Th1 and Th2 cells play in IDDM pathogenesis is not known. Earlier studies indicated that Th1 cytokines promote and Th2 cytokines protect from the diabetes progression (Rappoport *et al*, 1993; Pennline *et al*, 1994). Progression and onset of T1DM is due to an altered balance between Th1 and Th2 cells (Almawi *et al*, 1999).

IL-10 is a pleiotropic Th2 cytokine that is usually considered to have a role in the down regulation of cell mediated and cytotoxic inflammatory responses, thus being a potent anti-inflammatory mediator. It has been suggested that Th2 induced component of anti-ß cell immunity is mediated principally by IL-10 (Lee *et al*, 1996). Approximately 75% of the variation in IL-10 production appears to be genetically determined (Westendorp *et al* 1997). The gene encoding IL-10 has been mapped to chromosome 1q. We thus hypothesized that IL-10 gene polymorphism may participate in susceptibility to or clinical presentation of type-1 diabetes. The information on population structure of Abbottabad in relation to type-1 diabetes and IL-10 gene promoter region polymorphism was lacking. In the present study, we evaluated the association of -592 A in *IL10* to type-1 diabetes patients of Abbottabad.

Materials and methods

Ninety three unrelated individuals including 49 men and 44 women with age ranged from 18 to 70 years and with diagnosis of type-1 diabetes were recruited for this study. Ninety one healthy controls with no family history of diabetes were selected from Abbottabad City, Ayub Medical Complex. All these individuals were of Pakistani ethnicity and had a similar environmental, social background. These were resided in the same urban area. Type-1 diabetes patients were diagnosed following the method of American Diabetes Association (ADA) specially developed for type 1 diabetes (Synonymous 1997). The SNP among the control were tested for Hardy–Weinberg equilibrium. $\chi 2$ test was used to detect the differences in allele and genotype frequencies between controls and diseased individuals. It was considered statistically significant with P values <0.05.

IL-10 Gene Promoter Polymorphism

The targeted 5'-flanking region of the IL-10 gene, with -1351 to +19, was amplified by polymerase chain reaction (PCR). The template DNA used in this study was isolated from peripheral blood. The PCR mixture of 50-µl volume contained 1 µmol/l of each oligo-nucleotide primer having (sense; 5'-GTTCCTCCCAGTTACAGTCT-3' and antisense; 5'-CTGTCTTGTGGTTTGGTTTT-3'), 25 µl master mix, 19µl distilled water, 50-mM KCl, 1-mM $MgCl_2$, 200 µmol/l of dNTPs, 0.25-U Taq polymerase and 50-ng DNA. The PCR conditions were: denaturation for1 minute at 94°C, followed by 35 cycles of 1 minute at 94°C, 1 minute at 55°C, and 1.5 minutes at 72°C. The elongation step was performed at 72°C for 5 minute. Restriction fragment length polymorphism (RFLP) was used to detect allelic polymorphism of the IL-10 gene promoter region at position -592. Following PCR, 10 µl of the amplicon was incubated for 2 hours with 0.5 U *RsaI* in a total volume of 15 µl at 37°C, and fragments were separated in polyacrylamide gel electrophoresis and polymorphism was visualized by staining the fragments with ethidium bromide and illuminated by UV.

Statistical analysis

Chi-square test was used for statistical analysis to detect the differences between groups. The results were considered statistically significant at $P < 0.05$ level of probability.

Results

We surveyed the human population of District Abbottabad, Pakistan for Interleukin-10 gene polymorphism and its relationship with type-1 diabetes**.** Ninety three patients and 91 healthy individuals were considered for the present study. All individuals were ethnic Pakistani. The clinical and other related attributes of the subject groups are given in Table 1. By using specific primers, a 1370bp monomorfic fragment was raised by PCR in both control and patients (Figure 1). To detect polymorphism in IL-10 gene promoter region at position -592, the PCR product was digested with restriction enzyme Rsa-1. After the restriction digestion, four fragments were detected: 8, 42, 469, and 851bp in control (Figure 2). However in diabetes patients, five fragments of 8, 42, 240, 469 and 611bp were yielded (Figure 3). Two new fragments of 240 and 611bp were detected in diabetes patients and these were absent in healthy subjects. There might be mutation in fragment of 851 bp as it was disappeared in diseased patients after restriction digestion and appearance of two new fragments could have role in the development of disease. Over all significant difference between type-1 diabetes patients and healthy subjects was observed.

Table 1. Clinical characteristics of human population of District Abbottabad.

Characteristics	Patients	Control
No. of individuals	93	91
Male	49	50
Female	44	41
Family history of Diabetes	80	40

Table 2. Distribution of allelic frequency of *IL10* gene among the human population of District Abbottabad.

Polymorphism (Genotype)	Patient No. (%)	Control No. (%)
AA	13 (14)	91 (100)
AC	43 (46)	-
CC	37 (40)	-
Allel carriage	56 (60)	
AA, AC	37 (40)	
CC		

Genotype: $x^2 = 0.007$; $P = < 0.05$.

Genotype and allelic distributions of the *IL10* -592 C/A polymorphism in type-1 diabetes patients and control subjects is presented in Table-2. There was no difference between observed and expected distributions of genotypes for the healthy individuals group, and therefore it was considered to be in Hardy-Weinberg equilibrium. Significant difference was observed in genotype distribution between groups indicating that the frequency of AC genotype may be related to the occurrence of type-1 diabetes. It is observed that diabetes patients were carriers of the *IL10* -592A allele (AA, AC genotypes) than healthy subjects ($X^2 = 0.007$, $P = < 0.05$). It is documented that there is an association between the presence of the A allele and the occurrence of type-1 diabetes.

Discussion

The aim of the current study was to investigate whether the promoter region of *IL10* gne *at* position -592, affect the development of Pakistani population with type-1 diabetes. We were able to analyze the Pakistani individuals for polymorphism of Interlukine-10 gene promoter region at position -592 and to correlate it with the type-1 diabetes and

assumed that Interleukin-10 gene is responsible for type-1 diabetes in Abbottabad District of Pakistan. Recent studies have also reported that *IL10* gene polymorphisms were associated with several inflammatory diseases. (Wang *et al*, 2011) reported that IL-10 concentration was significantly higher in Crohn's disease patients than in the controls and *IL10* polymorphisms were associated with increased patient serum at IL-10 levels. (Hudson *et al*,2011) reported that *IL10* genotypes were associated with systemic sclerosis-related autoantibodies and contribute to the etiology of systemic sclerosis. (Mizuki *et al*, 2010) performed a genome-wide association study for Behect's disease and identified *IL10* as a disease susceptibility gene. (Muraközy *et al*, 2001) investigated an association of *IL10* polymorphisms with sarcoidosis, however they could not find any significant differences. In an investigation conducted in Japan, the Japanese population did not exhibit significant differences in genotype and allel frequencies in IL-10 at position -592 between healthy individuals and T1DM patient (Tegoshi *et al*, 2002). The results of the studies of Caucasian population, in France and Spain did not showed any significant association of T1DM with different haplotypes or genotypes of IL-10 promoter polymorphisms. (Urcelay *et al*, 2004; Reynier *et al*, 2006). A genetic association between three SNPs in the IL-10 promoter region and T1DM has been reported in Japanese populations, showing that -819 and -592 polymorphisms were associated with age at onset of disease (Ide *et al*, 2002). It has been proposed that variable production of Th2 cytokines including IL-10 may impudence both the degree of ß-cell destruction and the age of clinical onset (Ide *et al*, 2002). This study is the first to demonstrate the association between the genetic polymorphism and occurrence of type-1 diabetes in Pakistani population.

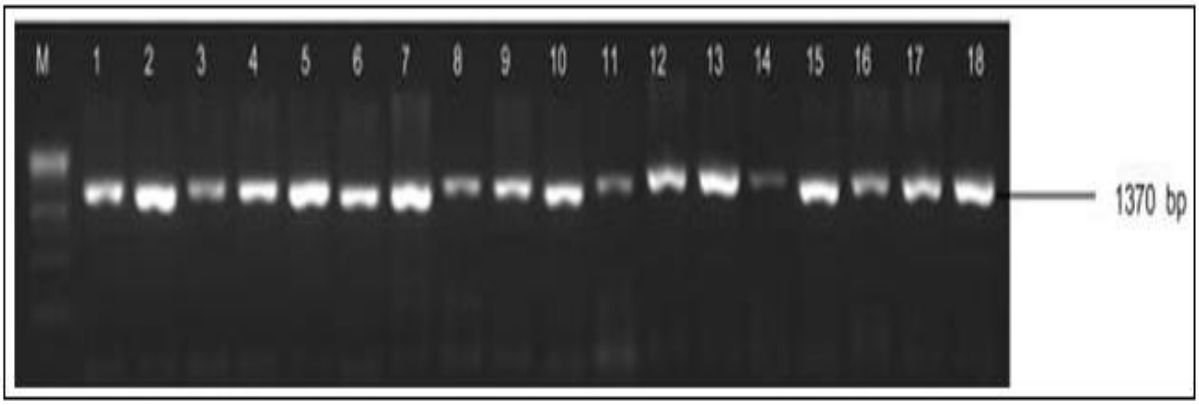

Fig. 1. (1 – 18). Amplification of 1370 bp fragment. Lane M = 1kb DNA ladder. Lane 1 – 9 = Control samples, Lane 10 – 18 = Diseased samples.

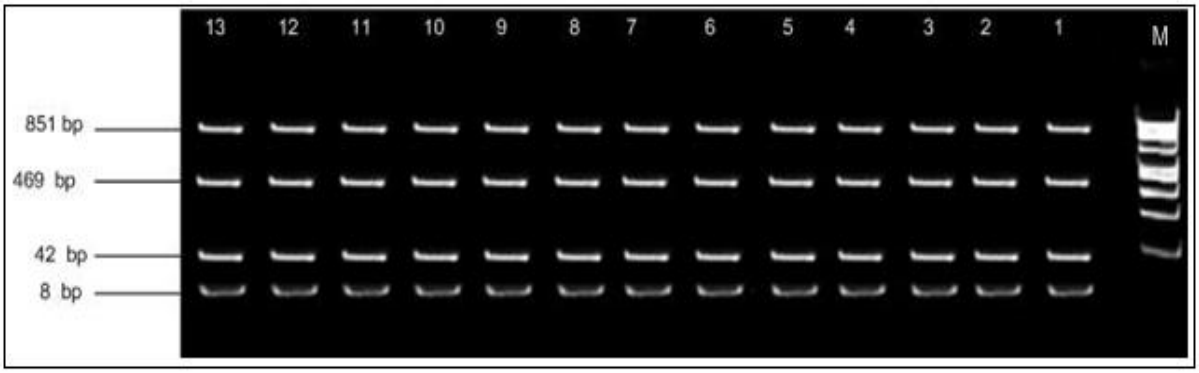

Fig. 2. (1 – 13). Restriction fragment length polymorphism of 1370bp fragment amplified by PCR in control subject. Lane M = DNA ladder. Lane 1-13, four fragments of 8bp, 42bp, 469bp, and 851bp were detected by agarose gel electrophoresis.

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