Letter to the Editor

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Association of CTLA-4 polymorphisms and autoimmune type-1 diabetes mellitus susceptibility in Pakistani population

Pakistan'da Yaşayan Kişilerde CTLA-4 Polimorfizmleri ve Otoimmün Tip-1 Diabetes Mellitus Duyarlılığı

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To the Editor,

Gene-gene interaction analysis is a potential strategy to help identify common disease susceptibility genes. Recently, evidence regarding the role of functional polymorphisms in negative immune-regulatory gene, cytotoxic T-lymphocyte antigen-4 (CTLA-4) has been reported in type 1 diabetes (T1D) [1]. CTLA-4 that maps to chromosome 2q33.34 is an important negative regulator of the immune system, whereas T cell proliferation is negatively regulated by CTLA-4. Several single nucleotide polymorphisms (SNPs) including +49 and –318 in the CTLA-4 exon 1 and promoter region are associated with certain autoimmune diseases including T1D [2, 3]. T1D affects many organs at the middle stage and shortens the life span of a patient, thereby imposing social and economic burden. TID is characterized by a loss of pancreatic beta-cell (B-cell) function and an absolute insulin deficiency. Since insulin is the primary anabolic hormone that regulates blood glucose level, a T1D patient thus requires a continuous supply of insulin for survival [4]. In Pakistan prevalence of T1D is very high, ranging from 7.6 to 11.0% and the rate of complications due to T1D to mother and new born are found to be higher, probably due to poor glycemic control [5]. Keeping in view the high burden of TID in our local population, a case control study was designed to analyze the impact of functionally important CTLA-4 polymorphisms on the outcomes of T1D susceptibility in Pakistani population belonging to Punjab province.

There are several functional polymorphisms in *CTLA-4* gene and its promoter. By carefully reviewing the available literature, the current study was conducted to explore the possible role of two functional polymorphisms in T1D onset in Pakistan. First polymorphism is in *CTLA-4* gene promoter region at position 318 (C to T; rs5742909) which effects the gene expression. The presence of allele C decreases the gene expression while allele T is associated with increased *CTLA-4* gene promoter activity. Second selected polymorphism is in exon 1 of *CTLA-4* gene at position 49 (A to G; rs231775). This polymorphism causes amino acid change from threonine to alanine in protein and results in inefficient CTLA-4 glycosylation and reduced cell surface expression.

The study included 100 T1D patients and 100 healthy controls. Restriction fragment length polymorphism

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Table 1: Association between CTLA-4 polymorphic allele, genotypes and autoimmune type-1 diabetes mellitus in Pakistan.

CTLA-4 locus	Patients		Controls		p-Value
	n (100)	Frequency (%)	n (100)	Frequency (%)	
Alleles					
+49A Allele	158	79	167	83.5	0.51
+49G Allele	42	21	33	16.5	
−318C Allele	151	75.5	154	77	0.49
−318T Allele	49	24.5	46	23	
Genotypes					
+49AA	66	66	73	73	0.79
+49AG	25	25	19	19	
+49GG	8	8	8	8	
−318CC	63	63	70	70	0.94
−318CT	23	23	17	17	
−318TT	14	14	13	13	

(RFLP) analyses was used to explore the CTLA-4 promoter polymorphism (–318 C/T) and exon 1 polymorphism (+49A/G) susceptibility with T1D as described previously [6]. The diagnosis of T1D was based on the blood glucose level according to the World Health Organization (WHO) diagnostic guidelines, clinical symptoms, absolute insulin-dependency and pancreas-specific autoantibodies. The C-peptide test was considered as confirmatory in the diagnosis of T1D for patients or to rule it out for controls. Other clinical characteristics, such as diabetic ketoacidosis at the initial presentation, age of onset, family history of T1D, pubertal status at the onset of diabetes and continuous dependence on insulin were also reviewed. All patients were non-obese and ≤15 years of age at onset. None of the controls had a family history of T1D.

Our results showed that the prevalence of CTLA-4 allelic/genotypic variants (+49A/G, –319 C/T) is similar in both studied groups and these polymorphisms are not significantly associated with T1D susceptibility (Table 1) in Pakistani population. Similarly no association was found with sex, weight, height, risk factors of diabetes or insulin treatment (data not shown). Existing knowledge about the association studies of these polymorphic variants with T1D onset in different ethnic groups showed inconsistent results. Variation in association between T1D and selected CTLA-4 SNPs in different populations could possibly be due to variation in *CTLA-4* gene expression linked to different haplotypes observed in different populations.

To the best of our knowledge, this is the first report from Pakistan to evaluate the role of CTLA-4 polymorphism on TID occurrence. Furthermore, we did not found any correlation between CTLA-4 polymorphic variants and TID, which may be due to small number of subjects. Although this study may well contribute to the literature, this may be considered as basic pilot study. Further exhaustive studies investigating the role of epistatic interaction of different important cellular players toward TID maybe required on a wider population size to obtain reliable results to investigate in detail.

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Ethical approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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