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Full Length Research Paper

TAP2 polymorphisms in Iranian patients with type I diabetes mellitus

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Type I diabetes (T1D) is an immune mediated disease characterized by immune destruction of insulin producing pancreatic beta cells. This disease is associated with the human major histocompatibility complex (HLA) region of the genome. The presentation of self peptides by HLA class I molecules is defective in individuals with this disease and both TAP1 and TAP2 are potential contributors to this defect. The aim of this study was to identify the correlation between TAP2 polymorphisms and T1D in Iran. Five known coding regions of TAP2 gene (sites 379, 565, 651, 665 and 687) were typed in a case-control study of 87 Iranian patients with T1D and 104 control subjects by using the amplification refractory mutation system (ARMS) PCR technique. The polymorphisms examined in codon 379 and 687 differed in frequencies between patients and controls and there is not any significant difference in frequencies between diabetic patients and control subjects in other three examined sites. TAP2 A was the most frequent allele in Iranian subjects (51.9% in healthy subjects and 47.1% in diabetic patients). The next frequent alleles in our subjects were TAP2 G and B and the frequencies of other alleles (TAP2 C, D, E and F) were usually lesser than 10%. TAP2 D allele was not present in our population and TAP2 E allele was not present in our diabetic patients. In conclusion, the polymorphisms examined in codon 379 and 687 differed in frequencies between patients and controls. We can consider these polymorphisms in all suspected individuals with diabetes under 30 years old.

Key words: Type I diabetes (T1D), transporter associated with antigen processing 2 (TAP2), human major histocompatibility complex (HLA), amplification refractory mutation system (ARMS), polymerase chain reaction (PCR).

INTRODUCTION

Type 1 diabetes mellitus (T1D), a common form of diabetes among children and young adults, originates from immune destruction of the pancreatic beta cells and is characterized by absolute insulin secretion deficiency, sudden onset of symptoms, presentation of ketosis and patient's dependency on exogenous insulin (Pociot and McDermott, 2002). This disease develops by contribution of different genetic and environmental factors (Tisch and McDevitt, 1996). Different forms of T1D have been described and many genetic studies have shown mapping of corresponding genes of this disease (Nagamine et al., 1997; Finnish-German APECED consortium, 1997; Verge et al., 1998; Dele´pineet al., 2000; Wildin et al., 2001; Bennett et al., 2001). Among many different genes linked to T1D presentation in humans, the most association has been reported from a single locus comes from several genes located in the MHC complex on chromosome 6p21.3, accounting for

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about 40% of the familial aggregation of this disorder (Pociot and McDermott, 2002). TAP genes are located between HLA-DQB1and HLA-DPA1 loci on human chromosome 6 and exhibit genetic polymorphisms. TAP1 and TAP2 proteins facilitate the transport of endogenous peptides across the membrane of the endoplasmic reticulum by an ATP-dependent route (Shepherd et al., 1993; Powis et al., 1993). In the endoplasmic reticulum, the peptides bind to the synthesized MHC class I molecules, and this binding stabilizes their conformation. These peptides then express with the class I molecules on the cell surface for presentation to CD8 + T cells. This process is necessary for the generation of cytotoxic T cells and tolerance to self for avoidance of auto reactivity and any change in the amino acid sequence of the antigen-binding cleft of HLA class I molecules may predispose to autoimmune diseases like T1D (Guerri et al., 2005). Polymorphisms in the TAP genes, both TAP1 and TAP2, may contribute to the statistically significant association between HLA class II genes and TID and association between TAP1 and TAP2 polymorphisms and insulin dependent diabetes mellitus have been described in several studies. In humans, at least eight polymorphisms in TAP2 gene have been identified (Zhang et al., 2002; Bahram et al., 1991; Powis et al., 1992; Szafer et al., 1994; Colonna et al., 1992; Moins-Teisserenc et al., 1994).

The aim of this study was to identify the allelic variants' distribution of TAP2 gene and estimate the correlation between TAP2 polymorphisms and T1D in Iran, because the coding TAP2 polymorphisms showed a strong association with type 1 diabetes in another populations (Pociot and McDermott, 2002; Guerri et al., 2005; Szafer et al., 1994; Colonna et al., 1992; Moins-Teisserenc et al., 1994). We examined the five known polymorphisms in the coding sequence with the use of amplification refractory mutation system (ARMS) polymerase chain reaction (PCR) analysis.

MATERIALS AND METHODS

A total of 87 patients with T1D and 104 healthy control subjects were studied. Control individuals were matched for age and sex with our patients. In all patients, diabetes was diagnosed in age less than 30 years. They had typical history of ketone positive hyperglycemia and required insulin treatment continuously from the time of diagnosis. Patients with malnutrition, pancreatitis and other immune mediated diseases were excluded. The control group did not have any history of diabetes or any other autoimmune diseases and this group was sex and age matched with patients. These individuals were selected from patients who attended Endocrinology clinics at Ghaem and Imam Reza hospitals of Mashhad for other complaints excepting diabetes mellitus.

The Research Ethics Board of the Mashhad University of Medical Sciences approved the study. Written informed consent was obtained from the subjects and from parents of subjects less than age 18 years. Genomic DNA was obtained after informed consent from T1D–affected subjects. DNA was isolated from peripheral blood lymphocytes with salting out method (Debomoy et al., 1991) from control subjects or individuals with T1D who attended Endocrinology Clinic at Ghaem and Imam Reza Hospitals of Mashhad. Polymorphic positions at position 379 (Val/Ile), 565 (Ala/Thr), 651 (Arg/Cys), 665 (Ala/Thr), and 687 (Stop/Gln) in TAP2 gene were analyzed with ARMS-PCR. Four primers were used for each site, one specific primer for each of the two variants and two other primers complementary to the flanking region as controls. The polymorphism is detected on the basis of the sizes of the PCR products. PCR was performed in a final volume of 20 µl containing 1 µl (100 ng) of DNA, 1.5 mM $MgCl_2$, 1.2 µg of each primer, and Taq DNA polymerase (0.5 U/20 µl). PCR conditions were as follows: 5 min at 95°C, 37 cycle at 94°C for 1 min, 58°C for 1 min (position 379), 64°C for 1 min (position 687) and 72°C for 1 min, and 10 min for final extension at 72°C. For other 3 sites, PCR conditions had some differences; 5 min at 95°C, 31 cycle at 94°C for 1 min, 64°C for 1 min (positions 565 and 665), 66°C for 1 min (position 651), and 72°C for 1 min, and 10 min for final extension at 72°C. PCR products were separated by 1.5% acryl amide gel electrophoresis and stained with ethidium bromide. Sequences of primers for TAP2 ARMS PCR are listed in Table 1.

Statistical analysis

Fisher's exact test was used to compare the distribution of each individual polymorphism between patients and controls. A P value of < 0.05 was considered statistically significant. For allelic variant' distribution, we used chi-squared test with Yates' correction if necessary. The delta value was calculated using this formula:

$$\Delta xy = Pxy - (PxPy)$$

where Pxy is the observed haplotype frequency for the X and Y allels and PxPy is the expected frequency of this haplotype based on the product of the frequencies of each allel X and Y (Hoarau et al., 2003).

RESULTS

We analyzed the previously defined TAP2 polymorphisms at codons 379 (ATA or GTA, Ile or Val), 565 (GCT or ACT, Ala or Thr), 651 (CGT or TGT, Arg or Cys), 665 (GCA or ACA, Ala or Thr), and 687 (CAG or TAG, Gln or Stop) by ARMS-PCR in Iranian individuals with T1D and controls. We studied 87 patients (31 men and 56 women, mean age = 17.3 years) and 104 healthy control subjects (46 men and 58 women, mean age = 22.5 years).

Among these five sites, only the polymorphisms examined in codon 379 and 687 was different in frequency between subjects and control group (P value = 0.013 and 0.001, respectively). The frequency of TAP2 polymorphisms in patients and control group in 3 other sites (565, 651 and 665) did not exhibit any significant differences in our study (P value = 0.704, 0.801 and 0.741 respectively) (Table 2).

Also, according to the previous studies and the most usual distribution of Allelic variation in TAP2 coding regions in other populations (Hoarau et al., 2003) and the data from our population we found that TAP2 A was the most frequent allele in Iran (51.9% in healthy subjects

Table 1. Sequence of primers used for ARMS-PCR analysis of TAP2 genotyping and sizes of the PCR products.

Site	Sequence (5'→ 3')	Primer name	Size (bp)
Codon 379 (ATA → GTA, Ile → Val)	GGAAGTGCTTCGGGAGATCCAGGATGCAGT	TAP2/379/C5	
	TTAAAAAGAACAAATAAAGCCCAAGGCC	TAP2/379/C3	581
	GAGACCTGGAACGCGCCTTGTACCTGCGCG	TAP2/379/Val	420
	ACAACCACTCTGGTATCTTACCCTCCTGAT	TAP2/379/Ile	220
Codon 565 (GCT → ACT, Ala → Thr)	CTCACAGTATGAACACTGCTACCTGCACAG	TAP2/565/C5	
	AGCTACAGGGACACGACCTTCACCACTAAG	TAP2/565/C3	460
	TGTTCTCCGGTTCTGTGAGGAACAACAGTA	TAP2/565/Thr	221
	ATCATCTTCGCAGCTCTGCAGCCCATAAAC	TAP2/565/Ala	298
Codon 651 (CGT → TGT, Arg → Cys)	AGAGGGAGGACGAAGGACCTACTAGTGGAA	TAP2/651/C5	
	GGCCTCAGTCCATCAGCCGCTGCTGCACCA	TAP2/651/C3	471
	CACCCCTTCAGCTGCAGGACTGGAATTACC	TAP2/651/Arg	195
	AGCAATCACCAGCACTGTGCGATCCCCTCA	TAP2/651/Cys	335
Codon 665 (GCA → ACA, Ala → Thr)	TTGGGGAATGGAATCCGGTGGTGTGAGGGC	TAP2/665/C5	
	GGTCCTGGAGACGCCCCTGAGAAGAGGG	TAP2/665/C3	462
	CAGTGCTGGTGATTGCTCACAGGCTGCAAA	TAP2/665/Thr	195
	CACCAGGATCTGGTGGGCGCGCTGAACTAC	TAP2/665/Ala	326
Codon 687 (TAG → CAG, Stop → Gln)	TCCAGCTGTGGCAGTACAGCCGGGAGAGAA	TAP2/687/C5	
	TCTCCATCGTGCCTGCAACTCAGGAACAGC	TAP2/687/C3	479
	AGGGCAAGCTGCAGAAGCTTGCCCAGCACC	TAP2/687/Gln	206
	CAGGCGGGAATAGAGGTCCTGTCCCTCATA	TAP2/687/Stop	332

Table 2. Frequencies of TAP-2 polymorphisms in patients with insulin-dependent diabetes mellitus and controls.

Site of polymorphism	IDDM patients		Controls		P value
	Number	Percent (%)	Number	Percent (%)	
379	74	85.1	3	2.9	0.001
565	4	4.6	3	2.9	0.704
651	0	0	1	1	0.801
665	50	57.5	54	51.9	0.741
687	15	14.4	5	5.7	0.013

and 47.1% in diabetic patients). The TAP2 G and B alleles were also present at a high frequency (19.5 and 16% in patients group and 21.1 and 16.3% in control group, respectively). The frequencies of other alleles (TAP2 C, D, E and F) were usually lesser than 10% in the patients. TAP2 D allele was not considered in our population and TAP2 E allele was not considered in our diabetic patients (Table 3).

DISCUSSION

T1D is induced by both environmental and genetic factors. The genetics of T1D has a long history of several studies evaluating responsible genes for association with this disease. Several genes have now been associated with T1D, providing idea that genetic evaluation is enough important in patients with this complex disease (Pociot and McDermott, 2002).

Among multiple studies two chromosomal regions have consistent and significant linkage to T1D. One of these regions is HLA. The main candidate genes for T1D, especially TAP genes are located between DP and DR of the HLA locus. Some studies showed that these genes cannot be a genetic factor in T1D presentation, because TAP1 alleles are found in the same frequency in T1D patients and healthy controls (Penfornis et al., 2002; Cano et al., 1995). In contrast with TAP1 gene, the

Table 3. Allelic variation in the TAP2 coding sequence in general population and diabetic patients.

Tap2 allele name		Normal Iranian population		Iranian patients with diabetes Type I	
WHO name	Local name	Number	Percent (%)	Number	Percent (%)
*0101	A	54/104	51.9	41/87	47.1
*0201	B	17/104	16.3	14/87	16
----------	C	1/104	0.9	4/87	4.5
----------	D	0	0	1/87	1.1
*0102	E	1	0.9	0	0
*0103	F	5	4.8	7/87	8
----------	G	22	21.1	17/87	19.5
*0202	---	0	0	0	0

unbalanced distributions of TAP2 were considered between diabetic patients and control groups (Jackson et al., 1995; Ronningen et al., 1993; Cucca et al., 1994; Caillat-Zucman et al., 1995). So, an association of TAP2 polymorphisms with T1D has been reported in the last studies. A single point mutation, generated by site-directed mutagenesis, in human TAP2 has also been shown to be sufficient to affect peptide transport specificity (Armandola et al., 1996). Although, two functional studies did not find any effect of human TAP1 polymorphism on peptide selectivity and transport (Obst et al., 1995; Daniel et al., 1997), the specific effect of the polymorphism at all codons of TAP2 on the peptide selectivity of the encoded transporter remained to be determined (Cucca et al., 1994).

In a study performed in Finland in 146 individuals with type I diabetes and 90 control subjects, there was absolute linkage disequilibrium for the polymorphisms at codons 604, 665, and 687 as well as the two downstream intronic polymorphisms in a 613-bp region of the 3′ portion of TAP2. The data were consistent with the existence of susceptibility haplotypes for T1D in the Finnish population consisting of DRB1*04 (*0401 and *0404), DQ8, and TAP2F (Rau et al., 1997). In another study in Germany sequence variants of TAP1 and TAP2 genes analyzed in 120 patients with IDDM and 218 random healthy German controls. TAP1*02011 and TAP2*0101 showed a positive association with IDDM (Penfornis et al., 2002).

We also used the ARMS-PCR to estimate the frequency of TAP2 polymorphisms that affect amino acids 379, 565, 651, 665, and 687. During the course of this study we observed linkage between amino acid variants at codons 379 and 687 as described previously in Iranian patients. The distribution of the codons 565, 651 and 665 polymorphisms did not differ significantly among diabetic subjects and control group. So, despite the absolute linkage between codons 665 and 687 previously detected in various populations but in our patients there was not detected.

TAP2 allele frequencies in our population exhibit significant differences with other populations. As previously reported in similar studies, TAP2 A was significantly higher in our T1D patients, but the frequency of other alleles in our subjects was different. TAP2 G and B alleles were present at a high frequency. The frequencies of other alleles (TAP2 C, D, E and F) were usually lower than 10% in our all subjects. TAP2 D allele was not considered in our normal population and TAP2 E allele was not considered in our diabetic patients.

Conclusion

This study had 3 important findings. First, in both patients and healthy controls TAP2 A was the most frequent allele. Second, the polymorphisms examined in codon 379 and 687 differed in frequency between patients and controls and third, TAP2 polymorphism may be associated with increased risk of T1D. There have been similar studies for other populations and in the current study indicated that the correlation exists in our study population which is Iranian diabetic patients. So, for diagnosis or further follow ups, 379 and 687 loci of this gene should be the center of focus.

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