Association Between PTPN22 Gene Polymorphism and Type1 Diabetes in Egyptian Population

اثبات الارتباط بين التباين الجيني للجين PTPN 22 ومرض السكري من النوع الاول في المجتمع المصري

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Abstract

The protein tyrosine phosphatase, nonreceptor 22 gene (PTPN22) maps at human chromosome 1p13.3 which encodes an important negative regulator of T-cell activation, lymphoid-specific phosphatase (Lyp). The PTPN22 gene has been shown to associate with a risk for multiple autoimmune diseases, including type1 diabetes (T1DM). This study aimed to analyze the association of three PTPN22 polymorphisms in Egyptian population .The single nucleotide polymorphisms (SNP) at positions -1123 (rs#2488457), 1858 (rs#2476601), and +2740 (rs#1217412) were genotyped in 60 persons with T1DM, and 60 control persons, all three SNPs were genotyping using two technique, allele specific PCR technique and restriction fragment length polymorphism – PCR (RFLP-PCR). The 1858 C/T did not show any significance differences between patients and control groups. (^{MC}P=1.0) whereas, respectively -1123 G/C and +2740 A/G were significantly associated with T1DM disease (P≤0.0001) and (P=0.012).These results suggest that the PTPN22 gene of SNPs polymorphisms were associated with type 1 diabetes in Egyptian population. The difference in the association of the aforementioned SNPs variants with T1DM among different populations may be attributed to the presence of multiple susceptibility alleles.

Keywords: Type 1 diabetes, protein tyrosine phosphatase, nonreceptor 22, polymorphism

الملخص

يقع الجين المشفر الى انزيم بروتين تايروسين فوسفاتيز نون رسبتر 22 على الكروموسوم p1 13.3 والمسؤول عن التنظيم السلبي لتشيط الخلايا التائية. أظهرت الدراسات ان الجين (PTPN22) له ارتباطات وثيقة مع خطر الاصابة ببعض امراض المناعه الذاتية,ومن ضمنها مرض السكري من النوع الاول. الهدف من هذه الدراسة هو تحليل الارتباط بين التباين الجيني للجين PTPN22 ومرض السكري من النوع الاول في عينة من المجتمع المصري. تم التحري عن النيكولوتيده الواحدة المتغيرة الشكل في الموقع (rs#2488457) 1123- ,1858(rs#2476601), and +2740 (rs#1217412), في 60 شخص من المرضى المصابين بمرض السكري من النوع الاول و60 عينه من مجموعة السيطره, تم استخدام تقنيتي PCR-RFLP و allele specific PCR لفحص التباين في النيكولوتيده الأحادية المتغيرة الشكل. عند اجراء الفحص لم يظهر هنالك اي ارتباط بين النيكولوتيده الأحادية المتغيرة الشكل 1858 C/T بالنسبة للمجموعه الضابطة ومجموعه المرضى مع مرض السكري من النوع الاول في المجتمع المصري حسب الدلالات الاحصائية (^{MC}P=1.0), بينما النيكولوتيده الأحادية المتغيرة الشكل لكلا -1123 G/C و +2740 A/G اثبتت انه هنالك ارتباط وثيق بينهم وبين مرض السكري من النوع الاول وفقا للدلالات الاحصائية (P≤0.0001) and (P=0.012) على التوالي. تقترح هذه النتائج المتعلقة بالجين (PTPN22) في النيكلوتيدات الاحادية المتغيره الشكل ان هنالك ارتباطا وثيقا بينها وبين مرض السكري من النوع الاول في المجتمع المصري. ويمكن ان تعزى هذه الاختلافات في النيكلوتيدات السالفه الذكر الى تغاير في صوره الجين (الاليل) للنيكلوتيده متغيره الشكل في المواقع السالفه الذكر في المجتمع المصري.

الكلمات المفتاحية: مرض السكري من النوع الاول, بروتين تايروسين فوسفاتيز نون رسبتر 22, تعدد الاشكال

Introduction

Type 1 Diabetes Mellitus results from β-cell destruction, usually leading to absolute insulin deficiency. Testing for islet-cell antibodies (ICA) or other autoantibodies (antibodies to glutamic acid decarboxylase [anti-GAD], insulin, and to the tyrosine phosphatase IA-2) in serum is useful in the diagnosis of T1DM where a positive result is indicative of immune-mediated or type 1A diabetes [1]. The association of *Protein tyrosine phosphatase non-receptor type 22 (PTPN 22)* was first described by Bottini [2] and quickly replicated by other studies [3,8]. It encodes a lymphoid protein tyrosine kinase (LYP) that is important in negative control of T-cell activation and in T- cell development [5, 9]. This polymorphism is the most potent after IDDM1 and IDDM2. The *LYP* gene, also termed *PTPN22*, *is a lymphoid tyrosine phosphatase* located on chromosome 1p13 [10]. It is of interest that *PTPN22* has an effect similar in magnitude to the insulin gene

polymorphism. Similar to *CTLA-4, PTPN22* is a T1DM susceptibility locus that is shared by several organ specific and systemic autoimmune diseases [2].

The aim of this study was to determine the association of the three *PTPN22* gene polymorphisms (SNP+2740 A ⁄ G rs#1217412 & SNP -1123 C ⁄ G rs#2488457 & SNP 1858 C ⁄ T rs#2476601) to the susceptibility to type 1 diabetes in the Egyptian population.

Materials and Methods

Study populations and sample DNA extraction

A total of 60 patients with type 1 diabetes (25 males and 35 females), mean age ±SD 11.2±3.7, and 60 healthy individuals (33 males and 27 females) mean age ±SD 27.2± 6.4 where used in this study.

Family history (25 positive/ 35 negative to family history, disease onset (years) mean±SD 5.3±3.5, were enrolled in this study and recruited at the El-Shatby University Hospital, Faculty of Medicine Alexandria University. Egypt. Patients diagnosed according to WHO criteria [11]. Patients had been diagnosed on the basis of classical clinical presentation, first-degree family history of diabetes, history of chronic diabetes complications, and treatment of diabetes. Healthy controls had no personal or first-degree history of diabetes and were free from T1DM. The Ethics Committees of the university and hospitals has approved the study, and informed consent was obtained from all participants.

An one ml of venous blood sample was collected in EDTA tubes from each individual (patient or healthy control) and was stored as whole blood at -20°C for subsequent DNA isolation. Genomic DNA was isolated from whole blood according to Sambrook *et al* [12].

Genotyping of PTPN22 gene polymorphism

Three SNPs (SNP+2740 A ⁄ G rs#1217412 & SNP -1123 C ⁄ G rs#2488457 & SNP 1858 C ⁄ T rs#2476601) in PTPN22 gene were genotyped among the participants groups in this study. The PTPN22 SNPs (SNP+2740 C ⁄ T rs#1217412, SNP -1123 C ⁄ G rs#2488457 and SNP 1858 C ⁄ T rs#2476601) was amplified by polymerase chain reaction (PCR) using allele specific PCR technique and restriction fragment length polymorphism –PCR (RFLP-PCR) as shown in Table (1). Four primers for each SNP (SNP+2740 C>T rs#1217412 and SNP -1123 C ⁄ G rs#2488457) (two allele specific primers, forward, control and common reverse primer) were designed based on the nucleotide sequence of a partial fragment (retrieved from the online dbSNP) of the gene containing the target SNP. The PCR product in (SNP 1858 C ⁄ T rs#2476601) was digested using an appropriate restriction enzyme (*RsaI*), the recognition site of *RsaI* is -GTAC-. after using forward primer 5́-ACTGATAATGTTGCTTCAACGG-3́ and reverse primer 5́-TCACCAGCTTCCTCAACC AC-3́ as shown in table 1 to amplify the SNP (1858 C>T rs#2476601), (the target SNP localized in the nucleotide sequence representing the recognition site of this restriction enzyme). The polymorphism was visualized by separating the DNA fragments on a 2% agarose gel after staining with ethidium bromide and illuminate by UV. To validate the PCR- allele specific and restriction fragment length polymorphism results as showed in figure (1, 2, and 3), all primers used in this study were newly designed using Primer Blast online programmed (http://www.ncbi.nlm.nih.gov/tools/primer-blast/).

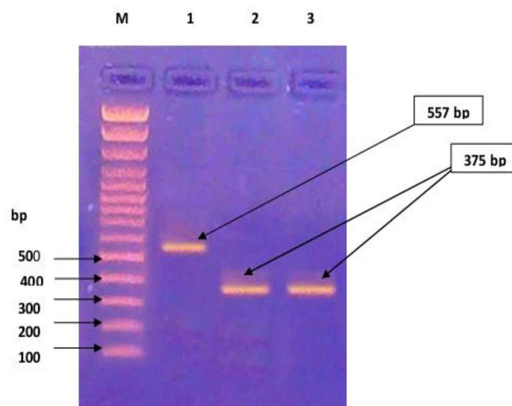

Fig. (1): **Shows 2% agarose gel electrophoresis for allele specific PCR for *PTPN-22* SNP +2740 A>G (rs#1217412). M: 100 bp DNA ladder from GeneDire*X*®. Lane1: PCR product upon using controls forward primer, Lanes 2 and 3: PCR products upon using allele specific A primer and allele specific G primer, respectively. Heterozygous genotype will give positive reaction upon using both allele specific primers. However, homozygous genotype will give positive.**

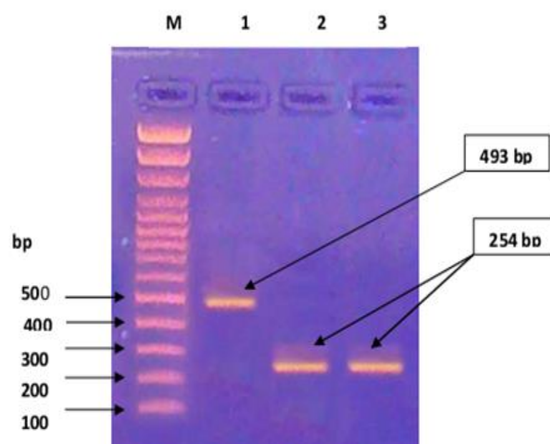

Fig. (2): **Shows 2% agarose gel electrophoresis for allele specific PCR for *PTPN* SNP -1123 C>G (rs#2488457). M: 100 bp DNA ladder from GeneDire*X*®. Lane1: PCR product upon using controls forward primer, Lanes 2 and 3: PCR products upon using allele specific C primer and allele specific G primer, respectively. Heterozygous genotype will give positive reaction upon using both allele specific primers. However, homozygous genotype will give positive reaction upon using only one of these allele specific primers.**

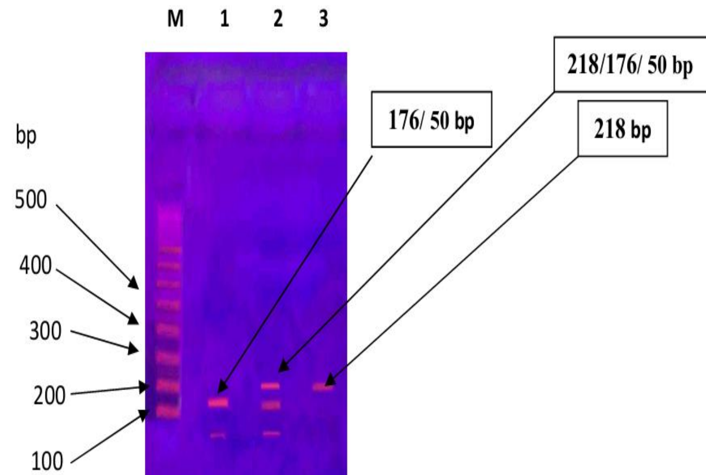

Fig.(3): Shows 2% agarose gel electrophoresis for RFLP-PCR for *PTPN-22* SNP 1858 C>T (rs#2476601). M: 100 bp DNA ladder from GeneDire*X*®. Lane 1 represents homozygous CC genotype as the C allele PCR products cleaved by Fast Digest® *RsaI* into 176 bp and 50 bp. Lane 2 represents heterozygous CT genotype as C allele PCR products cleaved by Fast Digest® *RsaI* into 176 bp and 50 bp while T allele PCR products were still 218 bp. Lane 3 represents homozygous TT genotype as the T allele PCR products cleaved by Fast Digest® *RsaI* still 218 bp.

Statistical analysis of data

Statistical analysis of the data was done to correlate genotype distribution and allele frequencies was performed by SPSS package version 11. The frequencies of alleles and genotypes between patient and control individual groups were compared using the chi-squared test (X2) and Monte Carlo test were used to test the significance of results the of quantitative variables. Odds ratio and 95% confidence interval (95% CIs) were calculated for different studied parameters. The confidence interval (CI) at 95% was used to describe the amount of uncertainty associated with the samples [13, 14]. A 95% confidence level means that 95% of the intervals would include the parameter. The significance of the results was taken at the P ≤ 0.05 level of significance.

Table (1): Primers sequences, PCR conditions, length of PCR products, restriction digestions and products of *restriction digestion*

SNPs	Primers sequences	PCR Conditions	Restriction Digestion	Size of PCR Products digestion products
PTPN22 **SNP 1858* C/T (rs#2476601)****	**Forward primer: 5́- ACTGATAATGTTGCT TCA ACG G -3́ Reverse primer: 5́- TCACCAGCTTCCTCA ACC AC -3́**	**An initial denaturation at 95°C for 5 min -Then, 30 cycles each cycle consisted of denaturation at 94°C for 60s, annealing at 50 °C for 30s and extension at 72°C for 30 s . -A final extension at 72°C for 10min.**	**PCR product was digested with *RsaI* enzyme . Total volume of digestion reaction mixture (30 µl) contained: 20µl of PCR product, 1 µl of *RsaI* (Fast digest) enzyme, 3 µl of 10X buffer, 1 µl of BSA and 5 µl nuclease free water. This mixture incubated at 37°C for 20 minutes**	**PCR product: 218 bp Restriction digestion products: 176bp &50 bp Allele C: 176bp and 50bp Allele T: 218 bp**
PTPN22 **SNP-1123 C/G (rs#2488457)****	**G-allele specific primer: F1: 5́- ATTGAGAGGTTATGC AAGCT G-3́ C-allele specific primer: F2: 5́- ATTGAGAGGTTATGC AAGCT C-3́ Forward control primer: 5́- GTTCAGATTAAGCAG TGTTCA G-3́ Common reverse primer: 5́-CCTGCA ATGTA ATG CTG GTA AA-3́**	**An initial denaturation at 95°C for 5 min -Then, 30 cycles each cycle consisted of denaturation at 94°C for 60s, annealing at 50 °C for 30s and extension at 72°C for 30 s. -A final extension at 72°C for 10min.**	**-**	**Allele C: 254 bp Allele G: 254 bp Control fragment : 494 bp**
PTPN22 **SNP+2740 A/G (rs#1217412)****	**A-allele specific primer: F1: 5́- CCTTTTGAAGTTTAT GTT TATGTA A-3́ G-allele specific primer: F2: 5́- CCTTTTGAAGTTTAT GTT TATGTA G-3́ Forward control primer: 5́- CCAGTTTTCCACAAC ATTTG-3́ Common reverse primer: 5́-GACCA AGGAATCCACCACCA -3́**	**An initial denaturation at 95°C for 5 min -Then, 30 cycles each cycle consisted of denaturation at 94°C for 60s, annealing at 50 °C for 30s and extension at 72°C for 30 s. -A final extension at 72°C for 10min**	**-**	**Allele A: 375 bp Allele G: 375 bp Control fragment: 557 bp**

Results and Dicussion

Results revealed that the allele and genotypic distributions did not significantly differ between the diseased population with T1DM and control (P≥0.05) for the SNP 1858 C / T (rs#2476601). On the other hand, there was a statistical significant differences between the patients and the control group (P=0.012*) for the SNP +2740 A / G (rs#1217412). Results also showed that AA genotype is a highly frequency between the patients and the control so the genotype AA consider as a risk factor with T1DM among patients and control. In addition, there was a statistical significant differences between the patients and the control (P≤0.0001*) for the SNP -1123 G ⁄ C (rs#2488457). Results showed that GG genotype frequency greater than CC and GC frequency among patients and control and the GG genotype consider as a risk factor with T1DM among the patients and the control as shown in Table (2).

Table (2): *PTPN22* gene polymorphism and allele frequencies among diabetic patients and control individual

Gene polymorphism	Cases		Control		Significance	OR (95% CI)
	No.	%	No.	%		
1858 C>T					^{MC}P=1.0	
CC	1	1.9	0	0.0		--
TT	12	22.2	12	22.6		0.0 (0.0-20.1)
CT	41	75.9	41	77.4		00 (0.0-18.1)
Allele frequencies						
C	60	0.13	60	0.11	----------------	
T		0.87		0.89		
-1123 G/C						
GG	47	78.3	16	26.7	X^2=54.900 P≤0.0001*	95% CI for difference: (-58.890308; 58.890308) *
CC	5	8.3	23	38.3		
GC	8	13.3	21	35.0		
Allele frequencies						
G	60	25.9	60	44.1	------------------	
C		74.1		55.9		
+2740 A/G						
AA	36	60.0	20	33.3	X^2=19.600 P=0.012*	95% CI for difference: (-37.837258; 37.837258) *
GG	10	16.7	14	23.3		
AG	14	23.3	26	43.3		
Allele frequencies						
A	60	55.9	60	55	--------------------	
G		44.1		45		

^{MC}P: Monte Carlo test X^2: Chi-Square test *significant at P≤0.05 -NA-: Not applicable

Present findings disagree with those of Tang. *et al.* [15] which prove that T1DM is associated with *PTPN22* 1858C/T gene polymorphism in Chinas population and targeting this promoter polymorphism should be dependent on ethnicity. Xuan. *et al.* [16] in ethnicity and sex-stratified

analyses, similar associations were found among Caucasians and within Caucasian male and female strata. The results of meta-analysis suggest that the *PTPN22* C1858T polymorphism was associated with susceptibility to T1DM among the Caucasian population, and males who carried the -1858T allele were more susceptible to T1DM than females. Additionally, Lavrikova. *et al*. [17] found that a given polymorphic marker was not statistically significant with T1DM in the transmission disequilibrium test, while analysis of the distribution of frequencies of alleles and genotypes showed the association with T1DM. Thus, the polymorphic marker C1858T of the *PTPN22* gene is associated with T1DM in Russian patients. On the other hand, Santiago. *et al*. [18] proved that the *PTPN22* 1858T allele is a T1DM susceptibility factor also in the Spanish population and it might play a different role in susceptibility to T1DM according to gender in early-onset T1DM patients.

Cinek. *et a*l. [19] proved that in two different Caucasian populations, the Czechs and the Azeri, no independent contribution can be detected either the +2740 3 ′-UTR SNP, and only the minor allele at *PTPN22* codon 620 contributes to the risk of autoimmunity.

Liu. *et al*. [20] investigated the relationship between the polymorphism of *PTPN22* gene and latent autoimmune 1 diabetes in adults (LADA) in Chinese Hans. They found that duration of diabetes, C-peptide, and GAD-Ab titer between the group carrying GC/CC and the group without allele C. The -1123G>C promoter polymorphism of *PTPN22* gene is associated with LADA in adult Chinese Hans. Tang. *et al*. [15] in a systematic review and meta-analysis for (+1858C/T, -1123G/C) polymorphisms with type 1 diabetes mellitus they detect no link for *PTPN22* -1123G/C polymorphism in Europe, America, and Asia. Kawasaki. *et al*. [21] proved that a regulatory SNP (−1123G/C; rs#2488457) was shown to be weakly associated with T1DM in Japanese and Koreans. The difference in the association of the aforementioned of above studies variants with T1DM among different populations may be attributed to the presence of multiple susceptibility alleles at the aforementioned genes variants, racial/ethnic differences in the distribution of these variants and multiple hypothesis testing.

Our study indicated that T1DM is associated with PTPN22 -1123 G/C and +2740 A/G gene polymorphism, and targeting this polymorphism should be dependent on ethnicity, while 1858 C/T polymorphism did not susceptibility locus for T1DM, further studies with well-designed among different ethnicity populations are required.

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