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Contribution of IL-10 (SNP -819 C/T and SNP-1082 G/A) polymorphisms variants to the risk of type 1 diabetes in Egyptian population

Ali Salim Al-shehmany[1], Ahmad A. El-Kafoury[2], Medhat A. Haroun[1], Amira M.Embaby [1]

[1]Department of Biotechnology, IGSR, Alexandria University,
[2] Department of Pediatric, Faculty of Medicine, Alexandria University

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Abstract: This study investigated whether interleukin- 10 (IL-10) gene promoter region polymorphisms are associated with susceptibility or clinical presentation of type 1 diabetes. The frequency of -1082G/A and -819C/T, polymorphisms was analyzed in 60 Egyptian patients with type 1 diabetes and in 60 healthy control subjects in a case-controlled study. However, the -819C/T not showed significance between patient and control (P=0.208) and 1082G/A were associated with T1DM disease (P<0.0001). These results suggest that the IL-10 gene promoter polymorphisms are associated with type 1 diabetes in Egyptian population.

Key words: IL-10 interleukin-10 polymorphisms, type 1 diabetes.

مساهمه التباين الجيني لجين الانترلوكين- 10 و (SNP -819 C/T SNP-1082 G/A) على مخاطر الاصابه بمرض السكري من النوع الاول في المجتمع المصري

علي الشحماني [1] ، احمد الكافوري [2]، مدحت هارون [1]، اميرة محمد[1]

[1] قسم التكنولوجيا الحيوية، معهد الدراسات العليا والبحوث، جامعه الاسكندرية، مصر
[2] زميل بقسم الاطفال، كلية الطب، جامعه الاسكندرية، مصر

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الخلاصة: تم اختيار عينة مكونة من 120 شخصا" ومقسمة إلى مجموعتين (60 من المرضى المصابين بمرض السكري من النوع الاول و 60 من الأشخاص الأصحاء كمجموعة ضابطة) تم التحري عن التباين الجيني في جين الانترلوكين (10) في المواقع التالية 1082G/A and -819C/Tوارتباطه بمرض السكري من النوع الاول وأظهرت النتائج بانه لايوجد هنالك ارتباط بين الموقع متعدد الاشكال 819C/T ومرض السكري من النوع الاول (P=0.208) حسب الدلالات الاحصائية بين مجموعة المرضى والمجموعة الضابطه بينما اظهرت النتائج انه يوجد ارتباط وثيق بين الموقع متعدد الاشكال 1082G/A ومرض السكري من النوع الاول بين مجموعة المرضى والمجموعه الضابطه حسب الدلالات الاحصائية (P<0.0001)، وهذه النتائج تشير الى وجود ارتباط وثيق بين الموقع متعدد الاشكال 1082 G/A لجين انترلوكين (10) ومرض السكري من النوع الاول في المجتمع المصري.

Introduction

Type 1 DM 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 may be helpful if establishing the diagnosis is important; a positive result is indicative of immune-mediated or type 1A diabetes (1). Several susceptibility loci involved in the disease development have been identified and were consistently replicated in different populations. These efforts contribute to a better definition of the molecular pathways leading to increased type 1 diabetes mellitus risk and this knowledge, in turn, may help in understanding the genetic basis of the disease (2,3).

Interleukin (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-β cell immunity is mediated principally by *IL-10* (4,5). The gene encoding *IL-10* has been mapped to chromosome 1q. Several polymorphic sites within the promoter region have been described, including two microsatellite polymorphisms and three biallelic polymorphisms at positions: −1082, −819, and −592 from the transcription start site (6,7). *IL-10* promoter SNP genotype and haplotype frequencies appear to exhibit different distributions according to ethnicity (8-10).

The aim of this study was to assess the contribution of This *Interleukin* IL-10 (SNP -819 C / T rs# 3021097 & SNP-1082 G / A rs# 1800896) polymorphisms, to the susceptibility to type 1 diabetes in the Egyptian population.

Materials and Methods

Study populations

A total of 60 type 1 diabetic patients (25 males /35 females) mean age ±SD 11.2±3.7, 60 healthy individuals (33 males / 27 females) mean age ±SD 27.2± 6.4, 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 participating universities and university hospitals approved the study, and informed consent was obtained from all participants.

Blood sampling was carried out, 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^{o}C for subsequent DNA isolation. Genomic DNA was isolated from whole blood according to Sambrook *et al* 1989 (12).

Genotyping of interleukin-10 gene polymorphism

Two SNPs (SNP -819 C / T rs# 3021097 & SNP-1082 G / A rs# 1800896) in IL-10 gene were genotyped among the participants groups in this study. The IL-10 (SNP -819 C / T rs# 3021097 & SNP-1082 G / A rs# 1800896) was amplified by polymerase chain reaction (PCR) using allele specific PCR technique as shown in Table 1. 4 primers for each SNP (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 polymorphism was visualized by separating the DNA fragments in a 2% agarose gel that was stained with ethidium bromide and illuminated by UV. To validate the PCR- allele specific results as showed in figure 1 and figure 2. All primers used in this study were newly designed using Primer Blast online programme http: // www. ncbi. nlm.nih.gov/tools/primer-blast/).

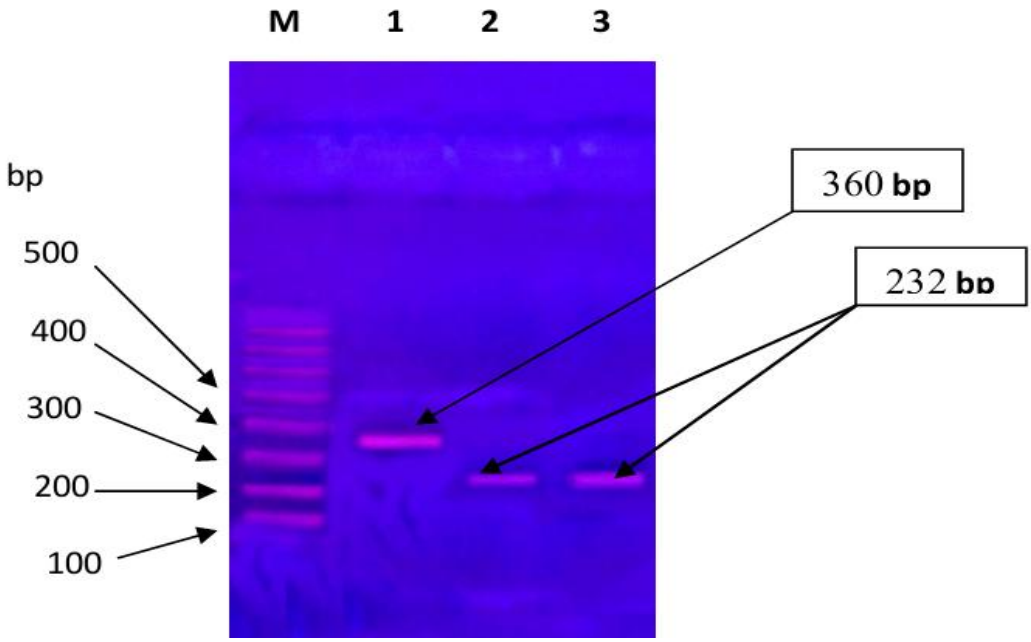

Figure 1: 2% agarose gel electrophoresis for allele specific PCR for *IL-10* SNP-819 C>T (rs #3021097). M: 100 bp DNA ladder from GeneDire*X*®. Lane1: PCR product upon using control forward primer Lanes 2 and 3: PCR products upon using allele specific C primer and allele specific T primer, respectively. 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.

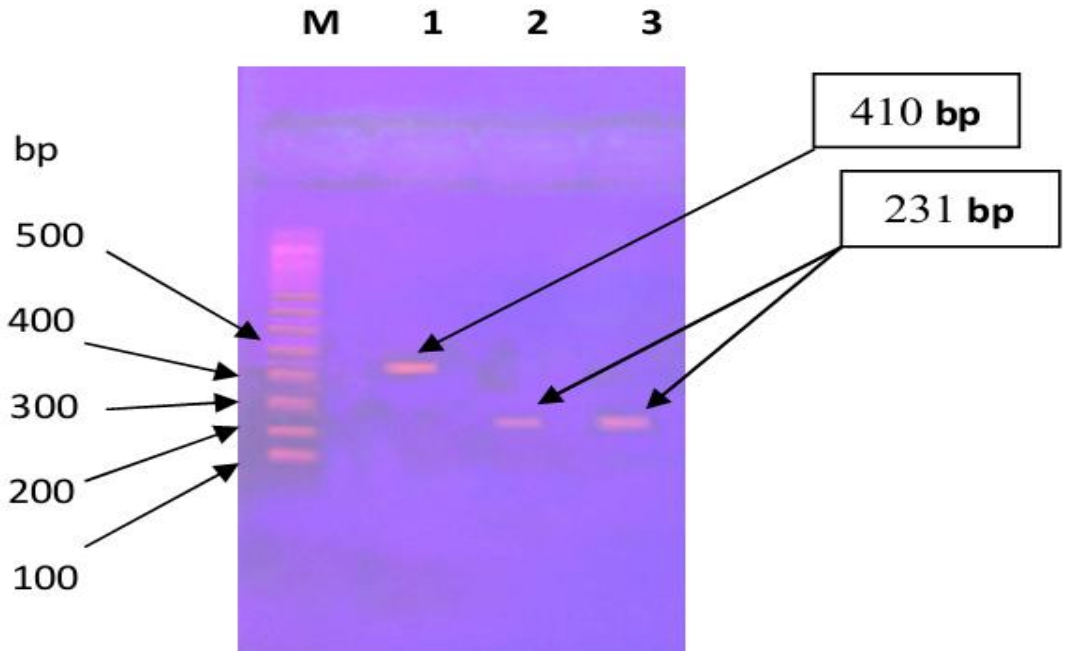

Figure 2: 2% agarose gel electrophoresis for allele specific PCR for *Il-10* SNP - 1082 G>A (rs# 1800896). M: 100 bp DNA ladder from GeneDire*X*®. Lane1: PCR product upon using controls forward primer Lanes 2 and 3: PCR products upon using allele specific G primer and allele specific A primer, respectively. 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.

Statistical analysis of data

Statistical analysis of data was done to correlate genotype distribution and an allele frequency was performed by SPSS package version 11. The frequencies of alleles, genotypes in different groups were compared using the chi-square test (X^2), t-test and Mann Whitney test were used to test the significance of results 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

SNPs	Primers sequences	PCR Conditions	Size of PCR Products digestion products
Il-10 SNP-819 C/T (rs #3021097)**	**T-allele specific primer:** F1: 5-CCC TTG TAC AGG TGA TGT AT **T**-3 **C-allele specific primer:** **F2:** 5-CCC TTG TAC AGG TGA TGT AT **C**-3 **Forward control primer:** 5-GAC TCC AGC CAC AGA AGCT-3 **Common reverse primer:** 5- GGATGT GTTCCA GGCTCC T-3	**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**: 232 bp **Allele T**: 232 bp **Control fragment**:360 bp
Il-10 SNP-1082 G/A (rs#1800896)**	**G-allele specific primer:** **F1**: 5- ACTACTAAGGCTTCTTTGGGA **G** -3 A-allele specific primer: **F2**: 5- ACTACTAAGGCTTCTTTGGA A -3 **Forward control primer:** 5- GACTCCAGC CAC AGA AGC T-3 **Common reverse primer:** 5- GGATGTGTTCCAGGC TCCT-3	-**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 G** : 231 bp **Allele A**: 231 bp **Control fragment:** 410 bp

Results and Discussion

Results revealed that the allele and genotypic distributions did not significantly differ between the two groups (P>0.05) for the SNP- 819 C/T (rs# 3021097). On the other hand, there was a statistical significant difference between the two groups (P<0.0001) for the SNP -1082 G/A (rs# 1800896). The genotype GA in *IL -10-1082* is protective from the disease as shown in table 2.

Table 2: IL -10 gene polymorphism and allele frequencies among diabetic patients and their control

Gene polymorphism	Cases		Control		Significance	OR (95% CI)
	No.	%	No.	%		
IL -10-1082						
GG	17	28.3	8	13.3	X^2=26.267	--
AA	22	36.7	4	6.7	P<0.0001*	2.6 (0.6-12.5)
GA	21	35.0	48	80.0		0.2 (0.1-0.6)*
allele frequencies						
G	60	0.47	60	0.17	----------	
A		0.53		0.83		
IL -10-819						
TT	15	25.0	22	36.7	X^2=3.14	--
CC	13	21.7	7	11.6	P=0.208	2.7 (0.8-9.9)
TC	32	52.3	31	51.7		1.5 (0.6-3.7)
allele frequencies						
T	60	0.36	60	0.42	----------	
C		0.64		0.58		

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

Studies conducted in France and Spain did not confirm any significant association of T1DM with different genotypes of *IL-10* promoter polymorphisms in Caucasians population (10,15). (Mohebbatikaljahi, H. *et al.* 2009) showed no link between T1DM and SNP-819 in *IL-10* (16).

Previous studies in Turkish population showed that at the *IL-10 −1082* (A/G) polymorphic site, the frequencies of GG genotype in patient and controls showed significant difference. This genotype was more prevalent in control group. Thus , the G allele is a protective allele and genotype 'GG' has a protective effect with a

significant P value for a negative association with T1DM (16). We can explain our results on the basis of clinical heterogeneity. It has been proposed that variable production of Th2 cytokines including *IL-10* may influence both the degree of β-cell destruction and the age of clinical onset (17).

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