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Genetic Association between Interleukin IL-18-137G/C and IL-18-607 C/A polymorphisms and Type 1 Diabetes in Egyptian population

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Abstract: Interleukin-18 also known as interferon-gamma inducing factor, Interleukin 18 is a cytokine that plays an important role in the T helper cell 1 response, by its ability to induce IFN- c production in T cells and natural killer cells. A functional variant of IL-18 gene has been reported as associated with type 1 diabetes (T1D). In the present study were analyzed two promoter single nucleotide polymorphisms (SNPs), at -607 (rs#1946518) and -137 (rs#187238) position, in 60 patient with type 1diabetes and 60 healthy individuals, both from Egyptian population. Allele and genotype frequencies of IL18 SNPs were compared in patients and controls .The genotype CC in -137 show significance according to the patient and their control (**P<0.004) while the SNP** – 607 don't showed any significance between patient and control (P=0.641), respectively. Our finding suggested an association between IL18 promoter SNP -137 and susceptibility toT1DM in Egyptian population.

Key words: interferon-gamma; Gene–gene interaction; IL-18; Polymorphism; Type 1 diabetes.

الارتباط الوراثي بين التباين الجيني للجين انترلوكين 18 -137G/C (and IL-18-607 C/A) ومرض السكري من النوع الاول في المجتمع المصري

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الخلاصة: انترلوكين 18, معروف ايضا" بالانترفيورن كاما, هو سايتوكين ويلعب دورا مهما في الاستجابة المناعية للخلايا التائية المساعده من خلال قدرتها على إنتاج IFN- c في الخلايا Tوالخلايا القاتلة الطبيعية. وهنالك عده تغيرات تم تسجيلها من خلال الدراسات بين انترلوكين 18 ومرض السكري من النوع الاول . وفي هذه الدراسة تم اختيار عينة مكونة من 120 شخص ومقسمة إلى مجموعتين (60 من المرضى المصابين بمرض السكري من النوع الاول و 60 من الأشخاص الأصحاء كمجموعة ضابطة في عينة من المجتمع المصري) . تمت المقارنة بين المجموعه الضابطه ومجموعه المصابين من حيث تردد التبيان الجيني وتردد الاليلات. اظهر التركيب الوراثي CC في الموقع 137- ارتباطا وثيقا مع داء السكري من النوع الاول وفقا للدلالات الاحصائية بين مجموعه المرضى والمجموعه الضابطه (P<0.004) بينما لم يظهر هنالك اي ارتباط بين الموقع 607 – ومرض السكري من النوع الاول بالنسبة للمصابين والمجموعه الضابطه وفقا للدلالات الاحصائية (P=0.641). ومن خلال النتائج وجد ان هنالك ارتباطا" وثيقا" بين انترلوكين 18(SNP-137) وقابلية الاصابة بمرض 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).

Interleukin (IL-18) serum levels are increased in the subclinical stage of T1DM in first degree relatives of T1DM patients. *IL-18*, which is predominantly secreted by activated monocytes/macrophages, is a pleiotropic cytokine involved in the regulation of innate and acquired immune response, playing a key role in autoimmune, inflammatory, and infectious diseases (2). T1DM in humans is also believed to be a Th1 lymphocyte–mediated disease, and both environmental and genetic factors play a role in its pathogenesis (3-5). The role of *IL-18* promoter polymorphisms in the predisposition to T1DM in humans. However, *IL-18* gene locus on chromosome 11q22.2– q23.3 has not been mapped by whole-gene scan studies as a region conferring major susceptibility to T1DM, therefore, role as a candidate for T1DM susceptibility gene, since the genetic association between *IL-18* and destructive insulitis has been suggested in the animal model of autoimmune diabetes (6-8).

The aim of this study was to assess the contribution of this Interleukin IL-18-137G/C, (rs#187238), IL-18-607C/A (rs#1946518) 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 (9). 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 (10).

Genotyping of interleukin-18 gene polymorphism

Two SNPs (SNP-607 C / A rs#1946518 and SNP -137G / C rs# 187238) in *IL-18 gene* were genotyped among the participants groups in this

study. The IL-18 (SNP-607 C / A rs#1946518 and SNP -137G / C rs# 187238) 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 /).

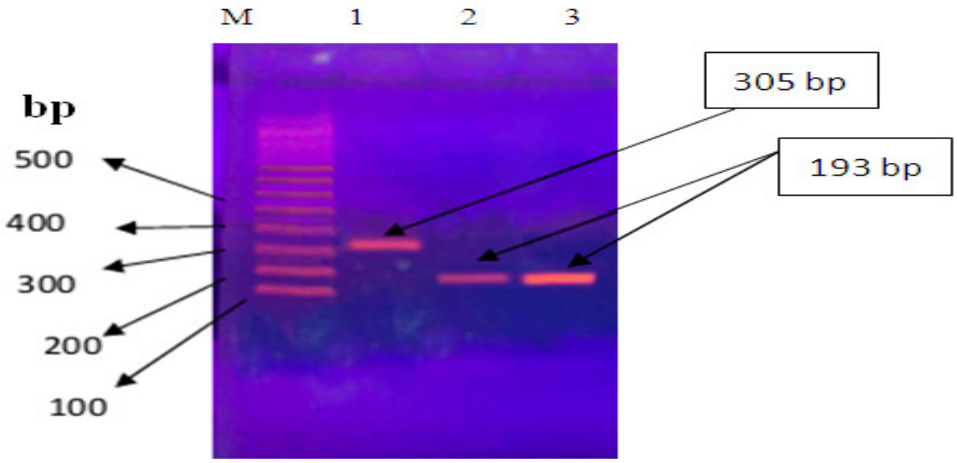

Figure1:Shows 2% agarose gel electrophoresis for allele specific PCR for *Il-18* SNP-607 C>A (rs#1946518). M: 100 bp DNA ladder from GeneDire*X*®. Lane1: PCR product upon using controls forward primer, Lanes 2 and 3: PCR products, upon using allele specific C primer and allele specific A primer, respectively. 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.

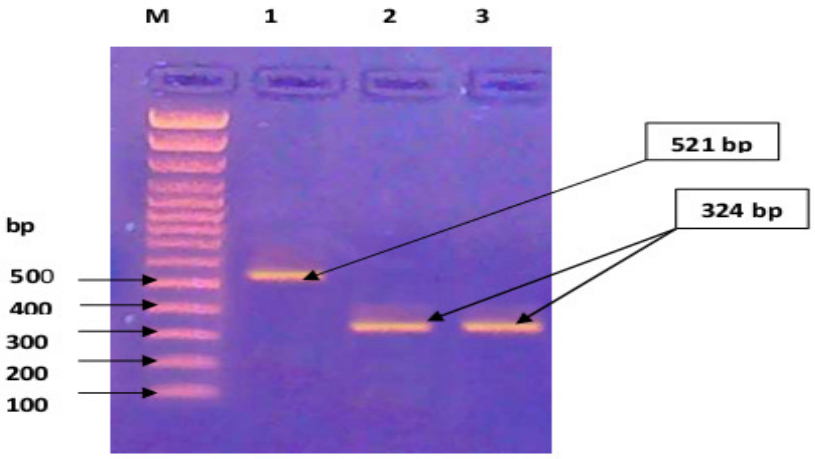

Figure 2:Shows 2% agarose gel electrophoresis for allele specific PCR for *IL-18* SNP -137G>C (rs# 187238). M: 100 bp DNA ladder from GeneDire*X*®. Lane1: PCR product upon using control forward primer. Lanes 2 and 3: PCR products upon using allele specific G primer and allele specific C primer, respectively. 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 allele frequencies was performed by SPSS package version 11. The frequencies of alleles, genotypes in different groups were compared using the chi-squared test (X2), t-test and Mann Whitney test were used to test thesignificance 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 (11,12). 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-18** SNP-607C/A (rs#1946518)**	C-allele specific primer: F1: 5-GTT GCA GAA AGT GTA AAA ATT ATT TC-3 A-allele specific primer: F2: 5-GTT GCA GAA AGT GTA AAA ATT ATT TA-3 Forward control primer: 5-GGT CAG TCT TTG CTA TCA TTC CA-3 Common reverse primer: 5-CCT CAT TCA GGA CTT CCC CTT-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: 193 bp -Allele A: 193 bp -Control fragment: 305 bp
Il-18* SNP-137G/C (rs# 187238)**	G-allele specific primer: F1:5-CCCCAACTTTTACGGAAGAAA AG-3 C-allele specific primer: F2:5- CCCCAACTT TTACGGAAG AAA A C-3 Forward control primer: 5- TGTAGGAATTACCCCAATAGG AC-3 Common reverse primer: 5- CTT CCCGAAGCTGTG TAG AC -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 52 °C for 30s and extension at 72°C for 30 s . -A final extension at 72°C for 10min.	Allele G: 324 bp Allele C: 324 bp Control fragment: 521 bp

Results and Discussion

Results revealed that the allele and genotype distributions did not significantly differ between T1DM patients and control Subjects (P>0.05) for the SNP 607C>A (rs#1946518). On the other hand, there was a statistical significant difference between the two groups (P<0.004) for the SNP -137G>C (rs# 187238) at the genotype and allele level. Present results suggest that the genotype CC is a risk factor for T1DM as shown in table 2.

Table 2 *: IL-18* gene polymorphism and allele frequencies among diabetic patients and their control

Gene polymorphism	Cases		Control		Significance	OR (95% CI)
	No.	%	No.	%		
IL -18 -607						
CC	11	18.3	15	25.0	X^2=0.78	--
AA	10	16.7	8	13.3	P=0.641	1.7 (0.4-6.9)
CA	39	65.0	37	61.7		1.4 (0.5-3.9)
allele frequencies						
C	60	0.27	60	0.32	----------	
A		0.73		0.68		
IL -18-137						
GG	15	25.0	25	41.7	X^2=10.900	95% CI for difference: (-23.095587; 23.095587)*
CC	32	53.3	12	20.0	**P<0.004***	
GC	13	21.7	23	38.3		
allele frequencies						
G	60	35.9	60	**60.9**	----------	
C		**64.1**		39.1		

X^2: Chi-Square test *significant at P≤0.05

Present results are in agreement partially with those of Novota *et al*. (2005) (13). Their results suggested that the two variants SNP 607C / A (rs#1946518) and SNP -137 G / C (rs# 187238**)** of *IL-18* gene are not associated with neither adult T1DM nor latent autoimmune diabetes in adults (LADA) susceptibility.

Conversely, Dong *et al*. (2007) (14) found that the CC genotype at position -607 in the promoter region of the IL-18 gene was significantly higher (risk factor) in Chinese Han children with T1DM than that in controls. While the AA genotype in -607 position could have a protective role for T1DM. Moreover, Eiji Kawasaki *et al*. (2004) (15) found that the distribution of the IL-18 gene genotypes at position –607 but not at position -137 was significantly different between T1DM patients control subjects (P=0.023). Present findings were partially in accordance with those of Mojtahedi *et al*. (2006) (16) in Iranian population. They found that there was no significant difference in the distribution of allele

and genotype at positions -137 and -607 of IL-18 gene between T1DM patients and control subjects without categorization of patients according to their age. On the other hand, present findings regarding allele and genotype at position -137 of IL-18 gene was in agreement with those of Massoud *et al*. (2009) (17) in Iranian population and those of Kretowski. *et al*. (2002) (18) in Polish population, suggested that an evidence of an association between type 1 diabetes and polymorphisms in the promoter of IL-18 gene. The difference in the association of the mentioned 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.

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References

1- AMERICAN DIABETES ASSOCIATION. (2008). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 31 Suppl 1, S55-60.

2- McInnes, I.B.; Gracie, J.A. ; Leung, B.P. ; Wei, X.Q. and Liew, F.Y. (2000). Interleukin 18: a pleiotropic participant in chronic inflammation. *Immunol Today*, 21, 312-315.

3- Patterson, C.C. ; Dahlquist, G. ; Soltesz, G. and Green, A. (2001). Is childhood-onset type I diabetes a wealth-related disease? An ecological analysis of European incidence rates. *Diabetologia*, 44 Suppl 3, B9-16.

4- Lonnrot, M. ; Korpela, K. ; Knip, M. ; Ilonen, J. ; Simell, O. ; Korhonen, S. ; Savola, K. ; Muona, P. ; Simell, T. ; Koskela, P. *et al.* (2000). Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study. *Diabetes*, 49, 1314-1318.

5- Kallmann, B.A.; Lampeter, E.F.; Hanifi-Moghaddam, P.; Hawa, M.; Leslie, R.D. and Kolb, H. (1999). Cytokine secretion patterns in twins discordant for Type I diabetes. *Diabetologia*, 42, 1080-1085.

6- Rothe, H.; Ito, Y. and Kolb, H. (2001). Disease resistant, NOD-related strains reveal checkpoints of immunoregulation in the pancreas. *J Mol Med (Berl)*, 79, 190-197.

7- Davies, J.L.; Kawaguchi, Y.; Bennett, S.T.; Copeman, J.B.; Cordell, H.J.; Pritchard, L.E.; Reed, P.W.; Gough, S.C.; Jenkins, S.C.; Palmer, S.M. *et al.* (1994). A genome-wide search for human type 1 diabetes susceptibility genes. *Nature*, 371, 130-136.

8- Concannon, P.; Gogolin-Ewens, K.J.; Hinds, D.A.; Wapelhorst, B.; Morrison, V.A.; Stirling, B.; Mitra, M.; Farmer, J.;Williams, S.R.; Cox, N.J. *et al.* (1998). A second-generation screen of the human genome for susceptibility to insulin-dependent diabetes mellitus. *Nat Genet*, 19, 292-296.

9- American Diabetes Association, Alexandria, Virginia. (2003). Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*, 26 Suppl 1, S5-20.

10- Sambrook, J.; Frisch, E.F. and Maniatis, T. (1989). Molecular cloning: A laboratory Manuel,2 nd edition. *Cold spring Harbor laboratory press, New York.*

11- Greenfield, B.; Henry, M.; Weiss, M.;Tse, S.M.; Guile, J.M.; Dougherty, G.; Zhang,

X.; Fombonne, E.; Lis, E.; Lapalme-Remis, S. *et al.* (2008). Previously suicidal adolescents: predictors of six-month outcome. *J Can Acad Child Adolesc Psychiatry*, 17, 197-201.

12- Szumilas, M. (2010). Explaining odds ratios. *J Can Acad Child Adolesc Psychiatry*, 19, 227-229.

13- Novota, P.; Kolostova, K.; Pinterova, D.; Novak, J.; Treslova, L.; Andel, M. and Cerna, M. (2005). Interleukin IL-18 gene promoter polymorphisms in adult patients with type 1 diabetes mellitus and latent autoimmune diabetes in adults. *Immunol Lett*, 96, 247-251.

14- Dong, G.P.; Yu, Z.S.; Liang, L.; Zou, C.C.; Fu, J.F. and Wang, C.L. (2007). IL-18 gene promoter -137C/G and -607C/A polymorphisms in Chinese Han children with type 1 diabetes mellitus. *Int J Immunogenet*, 34, 75-79.

15- Ide, A.; Kawasaki, E.; Abiru, N.; Sun, F.; Kobayashi, M.; Fukushima, T.; Takahashi, R.; Kuwahara, H.; Kita, A.;Oshima, K. *et al.* (2004). Association between IL-18 gene promoter polymorphisms and CTLA-4 gene 49A/G polymorphism in Japanese patients with type 1 diabetes. *J Autoimmun*, 22, 73-78.

16- Mojtahedi, Z.; Naeimi, S.; Farjadian, S.; Omrani, G.R. and Ghaderi A. (2006) Association of IL-18 promoter polymorphisms with predisposition to Type 1 diabetes. *Diabet Med,* 23, 235-239.

17- Massoud, A.; Bahai, N.S.; Massoud, M.;Salehi, E.; Massoud, A.H.; Vojgani, M. and Rajab, A. (2009). IL-18 gene polymorphism in type I diabetic patients: a case-control study. *Tehran University Medical Journal,* 67 ,20-24

18- Kretowski, A.; Mironczuk, K.; Karpinska, A. *et al*. (2002). Interleukin-18 promoter polymorphisms in type 1 diabetes. *Diabetes,* 51, 3347-3349.