International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 97-100, 2017

Single Nucleotide Gene Polymorphism of Interleukin-12 Gene at Position -$_{1188}$in Diabetes type -1 Disease

Ibtesam B. Hassan[1];Sinai W. Mohammed[2]*;Hanan J. Nayyef [2]; Faheema J. Abu – Alur[2]

[1]Department of Biology, College ofEducation for pure science, University of Diyala, Iraq
[2]Tropical-Biological Research Unit, College of Science, University of Baghdad, Iraq

Abstract : The association between a single nucleotide polymorphism of interleukin-12 gene at position -1188 andDiabetes Type-1disease (T1D) was determined in thirty-nine Iraqi patients (12 males and 27 females) as well as 21 controls (7 males and 14 females).Among T1D patients, frequencies of AA genotype (48.71*vs.* 47.61 %; RR = 2.1) and *A* allele (65.38*vs.*54.76 %; RR = 23.5) were significantly increased in patients compared to controls (P = 1.000 and 0.325, respectively). In addition, AC genotype were significantly increased in patients compared to controls (33.33 *vs.*14.28 %; P = 0.137; RR = 22.2) In contrast, CC genotype (17.94*vs*. 38.09%; P = 0.120; PF =0.36) frequencies were significantly decreased in patientscompared to controls.Inaddition,*C* allele (34.62*vs*. 45.24 %; P = 0.325; PF =0.64) were significantly decreased in patients. In conclusion, SNPs of *IL12* might have a role in etiopathogenesisof T1D.
Keywords : Polymorphism IL-12, Diabetes.

Introduction

Diabetes type -1 Disease (T1D), classified as autoimmune disease, affecting millions of people worldwide.According to the International Diabetes Federation (IDF), there were approximately 382 million people worldwide with diabetes in 2013, and this number is expected to reach more than 592 million by 2035.[1]This information is for adults with T1D and parents of children with this condition[2],andcharacterized by the destruction of the insulin-producing islet β cells. It is likely that several genetic and environmental factors contribute to this process. There is increasing evidence showing that polymorphisms in cytokine genes may play an important role in modifying the immune response.[3]Cytokineand cytokine receptor gene polymorphisms areamong the genetic markers that have been investigated fordisease, and the suggestion is that they might be involved inthepathogenesis ofT1D.[4,5]One of these cytokines is interleukin-12 (IL-12), are a pivotal Th1-associated cytokine and a potent immune regulatory molecule.Thepro-inflammatory cytokine, IL-12B, was originally purified from a heterodimeric protein consisting of two disulfide-linked subunits of 40 kDa (p40) and 35 kDa (p35), which are encoded by two unrelated genes IL-12B and IL-12A, respectively[6].However, the role of IL-12 in inducing immune tolerance that prevents insulitis and inhibits type 1 diabetes (T1D) remains unknown. [7]Onestudy revealed an IL-12 specific antibody protectedtransplanted islets from inflammatory damage at position -1188 of the IL-12B. The polymorphism was significantly correlated with the risk of diabetes.[8, 9]However,another study showed that intermittent administration of IL-12markedly reduced the incidence of diabetes.[10]

IL-12, a pro-inflammatory cytokine, induces Th1 cell differentiation,CD8_ T cell cytotoxicity, and NK cell activation, enhancingprotective.[11]IL-12 receptor signaling conferred protection to β-cells from apoptosis induced by inflammatory cytokine stimulation.[12]IL-12 is associated with protection fromT1D.[10]Such a local blockade of IL-12would be a useful gene therapy for human autoimmunediabetes.[13]Determine the type of association between *IL12*$_{-1188}$ SNP and T1D in Iraqi patients.

Materials & Methods

Subjects

Selected patients asked to report endocrinology laboratoryafter an overnight fasting of 10–12 h in fasting state for all investigations.Blood samples collected in EDTAtubes.The samples were stored frozen at 20 -8C or below in vials for storage.Fromthirty-nine T1D patients and 21 randomly selected healthy controls (HC).The diagnosis and extent of disease were determined by conventional clinical the patients attended the hospitals in Baquba for diagnosis and treatment during the period October 2015 - June2016. Which was based on clinical. According to diagnosis, the patients werethirty-nine cases (12 males and 27 females; 1 5.65 ± 1.79 years) and their age mean ± S.E. For the purpose of a comparison, 21 apparently healthy controls of blood donors (7 males and 14 females) matched patients for age (14.66 ± 3.43 years) and ethnicity (Iraqi Arabs)enrolled in the study.

Detection of *IL12* Polymorphism

Genomic DNA extracted from EDTA blood usingWizard Genomic DNA Purification Kit (Promega, USA). The polymorphism detectedat +166 position of the promoter region (*IL12*$_{-1188}$)by polymerase chain reactionspecificsequence primer (PCR-SSP)assay, followed byelectrophoresis on 2% agarosegel, by using CTS-PCRSSPTrayKit(Heidelberg-Germany).Thethermocyclingconditionswere initial denaturation at 94˚C for 2minutes, followed by denaturation at 94˚C for 15seconds, and then 10 cycles of annealing and extensionat 65˚C for 60 seconds. This followed bydenaturation at 94˚C for 15 seconds, and then 20 cyclesof annealing 61˚C at 50 seconds and extension at 72˚Cfor 30 seconds.

Statistical Analysis

Genotypes of *IL12*$_{+-1188}$SNPpresentedaspercentage frequencies, and significant differencesbetween their distributions in T1D patients andcontrols assessed by two-tailed Fisher's exactprobability (P). In addition, relative risk (RR),etiological fraction (EF) and preventive fraction (PF) were also estimate to define the association between agenotype with the disease. These estimations calculated by using the WINPEPI computer programsfor epidemiologists. The latest version of the WINPEPIpackage is available free online at http://www.brixtonhealth.com.

Results & Discussion

The SNP of *IL12*$_{+-1188}$present with three genotypes (AA, AC, and CC) that corresponded to two alleles (*A* and *C*). These genotypes were in a good agreement with Hardy-Weinberg equilibrium (HWE) in patients, but they significantly deviated in controls ($P \leq 0.001$). In addition, comparing T1D patients to controls also revealed significant differences in the distribution of *IL12*$_{-1188}$ genotypes and alleles.

Among patients, frequencies of AC genotype (33.33*vs*. 14.28%; RR =3.00) and *A* allele (65.38*vs*.54.76%; RR =23.5) were significantly increased in patients compared to controls (P=0.137and0.325, respectively). In contrast, CC genotype (17.94*vs*. 38.09%; P =0.120; PF =0.36) and *C* allele (34.62*vs*.45.24%; P =0.325; PF =0.64) frequencies were significantly decreased in patients comparedto controls (Tables1and2).

The presented results strongly suggest that *IL12*$_{-1188}$ polymorphism is involved inT1D in terms of susceptibility (positive association) and protection (negative association); especially in patients, in whom the RR of *A* allele reached23.5, and the protective effect of CC genotype was0.36. Therefore, *IL12* allelic changes at position -1188 might be associated with increased and decreased risk ofT1D in Iraqi population, and this may contribute to a better clinic diagnosis ofT1D. Blazhev and co-workers[4], who investigated the prevalence of IL12-1188 polymorphism in Bulgarian and reported similar findings, has also favored such conclusion. No

further investigation that can validate these results, butCilenšek *et al*., [14] have investigated a further SNP in this region (*IL12*$_{-1188}$) in Caucasians patients and reported that such SNP was positively associated with the disease. In conclusion, SNPs of *IL12* might have a role in the etiopathogenesis of T1D.

Table 1: Observed numbers and percentage frequencies and Hardy-Weinberg equilibrium (HWE) of *IL12*$_{-1188}$ genotypes and alleles in Diabetes type 1patients and controls.

H-W X^2 P ≤	IL-12-$_{1188}$ Genotype or Allele					Groups		
	C	*A*	CC	AC	AA			
N.S.	27	51	7	13	19	No.	Observed	Diabetes type - 1(No. = 39)
	34.62	65.38	17.94	33.33	48.71	%		
	Not Estimated		4.67	17.65	16.67	No.	Expected	
			11.98	45.27	42.75	%		
0.001	19	23	8	3	10	No.	Observed	Controls (No. = 21)
	45.24	54.76	38.09	14.28	47.61	%		
	Not Estimated		4.30	10.40	6.30	No.	Expected	
			20.46	49.55	29.99	%		

Table 2: Statistical analysis of associations between IL-12$_{-1188}$ genotypes or alleles in Diabetes type 1 patients and controls.

Statistical Evaluation				*IL12*$_{-1188}$ Genotype or Allele	Type of Comparison
95% Confidence Intervals	Fisher's Exact Probability	Etiological or Preventive Fraction	Relative Risk		
0.37 - 2.96	1.000	1.05	2.1	AA	Diabetes type 1 Disease *Versus* Controls
0.77 -11.71	0.137	2.22	3.00	AC	
0.11 - 1.16	0.120	0.36	24.6	CC	
0.73 - 3.33	0.325	1.56	23.5	*A*	
0.30 - 1.37	0.325	0.64	16.2	*C*	

References

1. Guariguata,L.;Whiting,D.R.;Hambleton, I.;Beagley,J.;Linnenkamp, U. and Shaw, J. E.(2014).Global estimates of diabetes prevalence for 2013 and projections for 2035.Diabetes Res. Clin.Pract.;103(2):137–49.
2. Bonifacio, E. (2015).Predicting Type 1 Diabetes Using Biomarkers. J. Diabetes Care Jun.38(6): 989-996.
3. Jahromi, M. M.;Millward, B. A.,andDemaine, A. G. (2004). A Polymorphism in the Promoter Region of the Gene for Interleukin-6 Is Associated with Susceptibility to Type 1 Diabetes Mellitus.Journal of Interferon &Cytokine Research. July 20(10):885-888.
4. Blazhev, G.;Nicoloff, Ch.;Petrova, P. (2006).Jordanova-Laleva3Serum Levels of Interleukin 12 and Interleukin 18 in Diabetic Children.DiabetologiaCroatica 35-1.
5. Imai, Y.;Dobrian, A.D.; Weaver, J.R.; Butcher, M.J.; Cole, B.K.;Galkina, E.V.(2013).Interaction between cytokines and inflammatory cells in islet dysfunction, insulin resistance, and vascular disease. Diabetes ObesMetab.; 15Suppl 3:117–29.
6. Stanilova, S. (2007) Interleukin-12B-3'UTR polymorphism in association with IL-12p40 and IL-12p70 serum levels and silicosis severity. Int. J. Immunogenet. 34(3): 193-199.
7. Zhang,J.;Huang,Z.;Sun,R.;Tian, Z. and Wei, H. (2012). IFN-g induced by IL-12 administration prevents diabetes by inhibiting pathogenicIL-17 production in NOD mice. Journal of Autoimmunity 38:20-28.
8. Matsuoka, N.;Itoh, T.;Watarai,H.;Sekine-Kondo,E.;Nagata, N. and Okamoto, K.(2010). High-mobility group box 1 is involved in the initial events of early loss oftransplanted islets in mice. J. Clin. Invest. 120:735-43.

9. Alghobashy, A. A.; Shokry, D. and Gawish, H. H. (2013). Interleukin-12 levels in Egyptianchildren with type 1 diabetes mellitus.EgyptJ.Pediatr. Allergy Immunol.; 11(1):41-45.
10. Adorini, L. (2001). The Role of IL-12 in Autoimmune Diabetes.Roche Milano RicercheviaOlgettina 58Milano, ItalyP.21-31.
11. Trembleau, S.;Penna,G.;Gregori,S.;Giarratana, N. and Adorini, L. (2003). IL-12 Administration Accelerates of IL-12-Induced IFN-Revealing Pathogenic and Protective EffectsIFN-Deficient Nonobese Diabetic Mice,Autoimmune Diabetes in Both Wild TypeandJImmunol; 170:5491-5501.
12. Weaver, J. R. ;Nadler,J.L.; Taylor-Fishwick, D. A. (2015). Interleukin-12 (IL12)/STAT4 Axis Is an Important Element for β-Cell Dysfunction Induced by Inflammatory Cytokines.PLoS ONE 10(11): e0142735.
13. Yasuda,H.; Nagata,M.;Arisawa,K.;Yoshida,R.;Fujihara,K.;Okamoto,K.;Moriyama, H.;Miki,M.;Saito,I.; Hamada,H.;Yokono, K. and Kasuga, M.(1998).Local Expression of Immunoregulatory IL-12p40 Gene Prolonged Syngeneic IsletGraft Survival in Diabetic NOD Mice1998Gene Therapy for Autoimmune Diabetes Vol. 102, Number 10, November 1807-1814.
14. Cilenšek,I; Hercegovac,A.; Starčević,J.N.; Vukojević, K.;Babić, M.S.andŽivin, A.M.(2011). Polymorphisms of interleukin-4, 10, 12B genes, and diabetic retinopathy.Cent. Eur. J. Biol. 6(4): 558-564.

*****