The Association Between IL-2 Gene (RS2069763) Single Nucleotide Polymorphism and Type 2 Diabetes Mellitus in Iraqi Patients

Maysaa Kadhim Al-Malkey
Tropical-Biological Research Unit, College of Science, University of Baghdad, Baghdad, Iraq

Abstract
This research attempts to find the association between single nucleotide polymorphism (SNP) of IL2+166 gene (rs2069763) and type 2 diabetes mellitus (T2DM) in a sample of Iraqi patients. A total of 44 patients and 55 apparently healthy volunteers were genotyped for the SNP using polymerase chain reaction test. Three genotypes (GG, GT, and TT) corresponding to two alleles (G and T) were found to have SNP. Both study groups’ genotypes had a good agreement for the analysis of Hardy-Weinberg Equilibrium. The results revealed increased frequencies between the observed and expected GG and TT genotypes and IL2+166 SNP T allele in T2DM patients (40.9 vs. 40.0 %; OR = 1.04; 95% CI, 0.47 - 2.31), whereas the values in the control group were 11.4 vs. 9.1 %; OR = 1.28; 95% CI, 0.35 - 4.68. Nevertheless, both variations did not reach a significant level. In the Iraqi population, the IL2+166 SNP was not associated with T2DM and, therefore, no association with its etiopathogenesis was found.

Keywords: Diabetes mellitus; Interleukin-2; Single nucleotide polymorphism

دراسة العلاقة بين تعدد أشكال النيكليوتيدة المفردة لجين الإنترلوكين-2 (RS2069763) ومرض السكري من النوع الثاني في المرضى العراقيين

ميساء كاظم المالكي
وحدة الأبحاث البايثولوجية للمناطق الحارة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة
حاول هذا البحث العثور على ارتباط بين تعدد الأشكال النيكليوتيدية المفردة لجين الإنترلوكين-2 (RS2069763) وداء السكري النوع الثاني (T2DM) في عينة من المرضى العراقيين. تم التضحي عن الطرز الوراثية في ما مجموعه 44 مرضي و 55 شخصًا من الأصحاء ظاهريًا كمجموعة سيطرة من العراق لتعدد أشكال النيكليوتيدية المفردة لجين الإنترلوكين-2 باستخدام مقايضة تسلسل تفاعل البمرنة المتسلسل (PCR) ثم العثور على ثلاثة أشكال وراثية (GG, GT وTT) والانتماء إلى الأليلات T وG. أظهرت هذه المورثات توافق جيد مع تحليل هاردي وليندرغ للتوالد للمجموعتين الداخلة في الدراسة. SNP كنت النتائج مع زيادة في الترددات بين الأشكال الوراثية TT وGG المرسومة والمتوافقة بالأليل T لجين L2+166 في المرضى الذين يعانون من سكري النوع الثاني (40.9 % مقابل 40.0 % وOR = 1.04 % و 95% CI، 0.47 - 2.31) بينما كانت في مجموعة السيطرة (11.4 % مقابل 9.1 % وOR = 1.28 % و 95% CI، 0.35 - 4.68).

*Email: maysakadhim@uobaghdad.edu.iq
Introduction

Type II Diabetes Mellitus (T2DM) is an endocrinological condition resulting from increased secretion of insulin and/or irregular insulin behavior [1, 2]. Reduction of insulin level results in chronic abnormally high blood sugar and resistance to glucose [3]. Currently, diabetes epidemic is related to a gene-environmental affliction. Worldwide prevalence estimates suggest that 85-95 percent of T2DM occurred in the developing countries.

In a 2010 study, the prevalence was reported to be 6.4 percent, affecting 285 million people in the age group of 20-79 years worldwide. It is expected that 438 million people will be diabetic in the developing countries by 2030, reaching 70 percent, along with a rise of 20 percent in developed countries [4]. Due to ongoing insulin resistance development and ß-cell dysfunction, the pancreas is unable to produce enough insulin to overcome insulin resistance. As a result, about 85% of the T2DM population is obese, leading to resistance to insulin [5]. Interleukin-2 (IL2) is the major growth factor for T cells and its binding to its specific receptors on T-helper cells stimulates their proliferation and production of effective cytokines from these cells [6]. This cytokine is under genetic control on chromosome locus 4q27. Expression levels of cytokine genes are often related to SNPs leading to their irregularity, which may modify the disease and thereby may lead to development of various immunological diseases, including T2DM [7]. Correlation of IL2 genetic variation with systemic lupus erythematosus (SLE) [8] and Multiple sclerosis (MS) [9, 10] as well as the IL18 SNP in Rheumatoid arthritis (RA) [11] were investigated to reveal potential impact of genetic variation with the disease etiopathogenesis. Therefore, this research focuses on possible association between T2DM and genetic variation of IL2 +166 G/T in a sample of Iraqi population with T2DM.

Materials and Methods

Subjects

Forty four T2DM patients (19 males and 25 females) were enrolled and their age range was 28-77 years (50.5 ± 15.5 years). The Iraqi Health Ministry Ethical Committee approved the study. During the time from February to March 2019, the patients were referred for diagnosis and treatment to the National Center for Diabetes Care and Research / Al-Mustansiriya University. The assessment was made by the clinical consultant and was based on the updated diagnostic criteria by the American Diabetes Association (T2D ADA criteria, 2010) [12]. The study also included 55 apparently healthy control volunteers. The control subjects were matched with the patients for the race (Iraqis), gender (27 males and 28 females) and they were aged from 17 to 70 years (31.9 ± 14.9 years).

Gene polymorphism of IL2

The TonkBio Genomic DNA Extraction Kit (New Jersey, USA) was used to extract genomic DNA from EDTA blood samples. Following purity and concentration evaluation, it was subjected to specific sequence primer technique using polymerase chain reaction test (PCR-SSP). Two primers were designed (Forward: 5'-CTGGAGCATTCTGCTGGATT-3' and Reverse: 5'-ACTCTTTACCTCAGATGAGCTGCTA-3') for the genotyping of IL2 +166 G/T SNP (rs2069763) using the Geneious software, version 10.1.3.

The PCR reaction was conducted at a final volume of 20 μl, including 6.5μl of the master mix (GoTaq green, Promega®- USA), 1 μl of the forward primer (10 μM), 1 μl of the reverse primer (10 μM), 1.5 μl of DNA (50 ng), and 10 μl of nuclease free distilled water. The following steps were applied as PCR reaction conditions: an initial denaturation at 94°C for 5 min, then 35 cycles of denaturation at 94°C for 30 sec, followed by annealing at 63°C for 30 sec and extension at 72°C for 30 sec, and ended by a final extension at 72°C for 10 min. The amplified PCR fragments were then run on agarose gel electrophoresis (1.5% agarose at 5 v/cm² for 45 minutes).

Statistical analysis

The age was expressed as mean ± SD, and the normality, randomization and homogeneity were calculated for the age by using IBM SPSS computer program V25.0. Frequencies of allele and genotype were given as percentage. The genotype frequencies were first tested for agreement with Hardy-Weinberg equilibrium (HWE), then Pearson's Chi-square analysis was performed to evaluate the significance of differences between the observed and expected genotype frequencies (https://www.easycalculation.com/health/hardy-weinberg-equilibrium-calculator.php). The relation...
between \( IL2 \) SNP and T2DM was addressed in terms of odds ratio (OR) and the exact probability of two-tailed Fisher was evaluated as a significant difference [13]. WINPEPI version 11.65 software for epidemiologists was used (www.brixtonhealth.com) to achieve the latter calculations.

**Results and Discussion**

**IL-2+166 gene polymorphism**

Genetic variations of \( IL2+166 \) (G/T) (rs2069763) SNP investigated the change of \( G \) allele to \( T \) allele at the nucleotide position 15 of the forward DNA strand. This change occurred at chromosome 4:122456327 positions. They were genotyped by PCR with allele specific primer (PCR-ASP) technique [14, 15]. The \( IL2 \) gene PCR amplified products revealed a single band of 301bp on agarose gel electrophoresis (Figure 1).

![Figure 1](image)

Figure 1- \( IL2 \) SNP (rs2069763) PCR product run on 1.5% agarose at 5 v/cm^2 for 45 min revealing bands of 301 bp molecular size. (Lane L: DNA ladder 1000 bp; Lanes D1-D10 samples of T2DM patients; Lanes C1-C5 samples of controls).

Analysis of HWE indicated that there was significantly no difference between frequencies of \( IL2+166 \) (G/T) (rs2069763) SNP in T2DM patients for both the observed and expected genotype. Therefore; these genotypes revealed a good equilibrium agreement with the HWE analysis in patients and control groups (Table 1).

**Table 1-** Observed and expected genotype frequencies of \( IL2+166 \) SNP (rs2069763) in type 2 diabetes mellitus and control.

| \( IL2+166 \) SNP Genotype | Patients (N = 44) | Control (N=55) |
|---------------------------|------------------|----------------|
|                           | Observed         | Expected       | Observed       | Expected       |
|                           | N    | %     | N    | %     | N    | %     |
| GG                        | 18   | 40.9  | 22   | 40.0  | 23.6 | 42.9 |
| GT                        | 21   | 47.7  | 28   | 50.9  | 24.8 | 45.1 |
| TT                        | 5    | 11.4  | 5    | 9.1   | 6.6  | 12.0 |
| HWE (p-value)             | 0.761 (NS)       | 0.351 (NS)     |

HWE= Hardy Weinberg equilibrium; N= Absolute number; \( p \)= probability; NS= Non significant; (\( p \)-value > 0.05).

The homozygous genotype (GG) showed an increased frequency in patients compared with controls (40.9 vs. 40.0 %; OR = 1.04; 95% CI, 0.47 - 2.31). Similarly, mutant homozygous genotype
(TT) frequency was increased in patients (11.4 vs. 9.1%; OR = 1.28; 95% CI, 0.35 - 4.68). Nevertheless, there was no significant level of both variations (p > 0.05). Also, there was no significant increase in the frequency of mutant allele (T) in patients (35.2 vs. 34.5%; OR = 0.57 - 1.85; 95% CI, 0.73 - 3.44) as shown in Table-2.

**Table 2:** Frequencies of allele and genotype of $IL2_{+166}$ SNP (rs2069763) in type 2 diabetes mellitus and control.

| $IL2_{+166}$ SNP Genotype | Patients (N = 44) | Control (N=55) | OR   | 95% CI         | $p$-value |
|---------------------------|-------------------|----------------|------|----------------|-----------|
| GG                        | 18                | 22             | 0.04 | 0.47 - 2.31    | 1.000 (NS)|
| GT                        | 21                | 28             | 40.0 | 0.40 - 1.93    | 0.840 (NS)|
| TT                        | 5                 | 5              | 0.04 | 0.35 - 4.68    | 0.747 (NS)|
| G                         | 57                | 72             | 0.04 | 0.54 - 1.74    | 1.000 (NS)|
| T                         | 31                | 38             | 35.2 | 0.57 - 1.85    | 1.000 (NS)|

N= Absolute number; OR= Odds ratio; CI= Confidence interval; $p$= Two-tailed Fisher exact probability; NS= Non significant ($p$-value > 0.05).

Type 2 diabetes mellitus involves multiple metabolic dysfunctions resulting in hyperglycemia from decreased insulin production and increased insulin resistance, in addition to irregular glucagon metabolism and signaling pathways of insulin and beta-cells [16]. It is proposed that several interleukins contribute to T2DM's pathology and have impacts on the signaling pathways of insulin and the function of beta-cell [17]. In the current study, there is a lack of association between $IL2_{+166}$ G/T SNP and T2DM in the investigated Iraqi population. A study by Howson et al., (2011) in Ulm and the surrounding area, southwest Germany, revealed an increased risk of genetic variation of $IL2_{+166}$ SNPs (rs2096763 and rs2096762). An association between $IL2$ (rs2069763) among 786 T2DM cases and 1,484 controls and its predisposition to adult-onset autoimmune diabetes was indicated [18], which contradicts our findings due to variations in sample size and ethnicity. A relevance association between $IL2$ (rs2096763) SNP and type 1 diabetes (T1DM) among white European ancestry was investigated by Howson et al., (2012) and revealed that the major allele G confers protection from T1DM, with an average of GG alleles at age of nine years old. $IL2$ gene expression is correlated to T1DM pathogenesis, added to that two $IL2$ receptor genes ($IL2_{RA}$ and $IL2_{RB}$) are both associated with type 1 diabetes [19]. It is suggested that $IL2$ gene variation has a strong correlation to the exacerbation of autoimmune types of diabetes mellitus with increasing age to older than 17 years [19], which was also confirmed by another two previous studies [20, 21].

To the best of our knowledge, $IL2_{+166}$ (rs2069763) genetic variations and its predisposition to T2DM as a risk factor in Iraqi population have not been previously demonstrated. Meanwhile, a study by Hamid and Shani (2018) investigated the association between IL-10 (-592A/C) gene polymorphism with the progression of T2DM in Basrah Province and revealed significant association between CA genotype with the risk of T2DM and that IL-10 (-592A/C) SNP contributes to the development of T2DM [22]. However, the impacts of $IL2$ (rs2069763) SNP on several diseases were highlighted, such as systemic lupus erythematosus [8], multiple sclerosis [9, 10], tacrolimus gravis [23], susceptibility to different cancer pathologies [24], in addition to its impact on serum level of IL2 among Iraqi Arabs [25].

**Conclusions**

This study results showed that GG genotype / T allele of $IL2_{+166}$ SNP is not an attributed factor for T2DM in this sample of Iraqi patients. In order to increase the statistical power for further investigations, the sample size is needed to be increased and different clinical courses for the disease could be implemented. Added to that, cytokine gene expression studies will be required with different diabetes mellitus clinical courses and associations to therapy response.
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References

1. American Diabetes Association, 2011. “Executive summary: standards of medical care in diabetes—2012. Diabetes care, 35(S1): S4–10.

2. Lyons, R.A., Benvenuti, L., 2016. Deposition and Distribution Factors for the Endocrine Disruptor, 4-Nonylphenol, in the Sierra Nevada Mountains, California, USA. J Environ Anal Toxicol, 6: 388.

3. Jahan, S., Fariduddin, M., Sultana, N., et al., 2015. Predictors of Post-Partum Persistence of Glucose Intolerance and Its Association with Cardio-Metabolic Risk Factors in Gestational Diabetes Mellitus. J Diabetes Metab, 6: 609.

4. Shaw, J.E., Sicree, R.A. and Zimet, P.Z. 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract, 87(1); 4–14. doi: 10.1016/j.diabres.2009.10.007.

5. Yamada, H., Suzuki, D., Kakei, M., and Kusaka, I. and Ishikawa, S. 2016. Close Association of Hypoadiponectinemia and Increased Insulin Resistance in Non-Obese Japanese Type 2 Diabetes with Visceral Adiposity. J Metabolic Synd, 5: 215.

6. Benary, M., Bendfeldt, H., Baumgrass, R. and Herzl, H., 2008. Modeling IL-2 gene expression in human regulatory T cells. In: Genome Inform. International Conference on Genome Informatics, 20: 222–30.

7. Saxena, M., Srivastava, N. and Banerjee, M. 2013. Association of IL-6, TNF-α and IL-10 gene polymorphisms with type 2 diabetes mellitus. Mol Bio reports, 40: 6271–79.

8. Lin, Y.J., Wan, L. and Sheu, J.J. 2008. G/T polymorphism in the interleukin-2 exon 1 region among Han Chinese systemic lupus erythematosus patients in Taiwan. Clin Immunol, 129: 36–39. https://doi.org/10.1016/j.clim.2008.05.011.

9. Al-Naseri, M.A.S., Ad’hiah, A.H. and Salman, E.D. 2019. The association between multiple sclerosis and genetic variations of TGFβ1 and IL2 genes in Iraqi patients. Meta Gene, 19: 253–7. doi:10.1016/j.mgene.2019.01.001.

10. Cavanillas, M.L., Alcina, A. and Ñez, C. 2010. Polymorphisms in the IL2, IL2RA and IL2RB genes in multiple sclerosis risk. Eur J Hum Genet, 18(7): 794–99. doi:10.1038/ejhg.2010.15.

11. Mahmood, A.S., Al-Kazaz, A.K.A. and Ad’hiah, A.H. 2018. Single Nucleotide Polymorphism of IL1B Gene (rs16944) in a Sample of Rheumatoid Arthritis Iraqi Patients. Iraqi J Sci, 59(2C): 1041–45. DOI:10.24996/ijs.2018.59.2C.7

12. Centers for Disease Control and Prevention, 2011. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention.

13. Ad’hiah, A.H. 1990. Immunogenetics studies in selected human diseases. Ph.D. Thesis, University of Newcastle upon Tyne.

14. Ye, J., Coulouris, G. and Zaretzkaya, I. 2012. Primer-blast: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics, 13: 13.

15. Abbas, A.H. and Melconian, A.K. 2018. The role of infectious agents and TLR-7, 9 gene polymorphisms as risk factors for systemic lupus erythematosus among Iraqi female patients. PhD thesis, Biotechnology Department, College of Science, University of Baghdad, Iraq.

16. Lončar, A., Blaslov, K., Bulum, T. and Duvnjak, L. 2015. The effect of GLP-1 analogues on lipid profile in type 2 diabetic patients—a retrospective observational study. Diabetol Croat. 44(2): 59–6.

17. Fève, B. and Bastard, J.P. 2009. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol, 5(6): 305–11. doi: 10.1038/nrendo.2009.62.

18. Howson, J.M.M., Rosinger, S. and Smyth, D.J. 2011. Genetic analysis of adult-onset autoimmune diabetes. Diabetes, 60(10): 2645–53. doi:10.2337/db11-0364.

19. Howson, J.M.M., Cooper, J.D. and Smyth, D.J. 2012. Evidence of gene-gene interaction and age-at-diagnosis effects in type 1 diabetes. Diabetes, 61(11): 3012–17. doi:10.2337/db11-1694.

20. Smyth, D.J., Plagnol, V. and Walker, N.M. 2008. Shared and distinct genetic variants in type 1
diabetes and celiac disease. *N Engl J Med*, **359**: 2767–77.

21. Fung, E.Y., Smyth, D.J. and Howson, J.M. *2009*. Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. *Genes Immun*, **10**: 188–191.

22. Hamid, S.M. and Shani, W.S. *2018*. The association of IL-10 (-592A/C) gene polymorphism with progression of Type 2 Diabetes Mellitus in Basrah Province-Iraq. *Iraqi J Sci*, **59**(2B): 819–26. *DOI:10.24996/ijs.2018.59.2B.1*

23. Shumei, Y., Yi, L. and Huanyu, M. *2019*. IL-2 gene polymorphisms affect tacrolimus response in myasthenia gravis. *Euro J Clinic Pharma*, **75**(6): 795–800.

24. Zhang, M., Tan, X. and Huang, J. *2016*. Association between two interleukin-2 gene polymorphisms and cancer susceptibility: A meta-analysis. *Onco Targets Ther*, **9**: 2181–92. *doi:10.2147/OTT.S94761.*

25. Ad’hiah, A.H., Al-naseri, M.A. and Ahmed, Z.A. *2019*. Cytokine gene variations and their impact on serum levels of IFN-γ, IL-2, IL-4, IL-10 and IL-12 among Iraqi Arabs. *Meta Gene*, **19**: 98–103. *doi:10.1016/j.mgene.2018.11.005.*