ASSOCIATION BETWEEN POLYMORPHISM IN CYTOTOXIC T LYMPHOCYTE ANTIGEN -4 GENE AND THE RISK OF RHEUMATOID ARITHRITIS IN IRAQI PATIENTS

Rawaa A. A.
Lecturer
Dept. Biology - Coll. Science- Mustansiriyah University
rawaaattar@uomustansiriyah.edu.iq

ABSTRACT

CTLA4 is well known that it is expressed in activated T-lymphocyte, and can be distinguished in cancer phenomena as well as many chronic diseases. This study was designed aiming to find a correlation between CTLA-4 and Rheumatoid Arthritis (RA). This research was carried out at Baghdad Teaching Hospital, Medical City, Baghdad, Iraq. In this study, 245 patients diagnosed with rheumatoid arthritis and 188 healthy volunteers' individuals (control group) were selected randomly. The diagnosis of the RA to both patients group and healthy individuals were characterized according to a certain protocols. The sequence of four (JO31, CT60, +49, and MH30 genotypes) were carried out using PCR-DGGE. The polymorphisms (SNPs) were genotyped for the control and the patients and the distribution of the genotypes was carried out according to HWE (P<0.05) for the two groups. The effect of two alleles on the susceptibility of RA, were investigated thoroughly, and there was a major association between the CT60, +49 alleles with the risk of RA (OR 1.43, 95%Cl (1.14-1.89), and the *P*-value 0.004 for CT60), while for +49 it was (OR 1.38, 95%Cl (1.04-1.77) and the P-value 0.009). There is a significant correlation between CTLA-4 genes and Rheumatoid Arthritis (RA) and both CT60 and +49 can be the main reason for RA, while JO31 and MH30 are not associated with CTLA-4.

Keywords: RA, CTLA-4, CT60, +49, genotype

مجلة العلوم الزراعية العراقية -2022 :53(2):252- 257 **عبد الجبار**

العلاقة بين تعدد الأشكال في جين مستضد الخلايا اللمفاوية التائية القاتلة وخطر الإصابة بالتهاب المفاصل الروماتويدي لدى المرضى العراقيين

رواء عبد الأمير عبد الجبار
مدرس
قسم علوم الحياة–كلية العلوم–الجامعة المستنصرية

المستخلص

يعبر عن جين CTLA4 في الخلايا التائية المنشطة، وهي معروفة ويمكن تمييزها في حالات السرطان وكذلك العديد من الأمراض المزمنة. تم تصميم هذه الدراسة بهدف إيجاد علاقة بين جين CTLA–4 والتهاب المفاصل الروماتويدي (RA). تم جمع العينات من مستشفى بغداد مدينة الطب، بغداد، العراق. اجريت هذه الدراسة على 245 مريضًا تم تشخيصهم بالتهاب المفاصل الروماتويدي، بينما كان الأفراد الأصحاء 188 تم اختيارهم عشوائيًا كمتطوعين. تم تشخيص التهاب المفاصل الروماتويدي لكل من مجموعة المرضى والأفراد الأصحاء وفقًا لبروتوكولات معينة. تم تنفيذ تسلسل أربعة أنواع وراثية (JO31 وCT60 و +49 وMH30) باستخدام PCR–DGGE. ولقد اجري تنميط (SNPs) للسيطرة والمرضى وتم توزيع الأنماط الجينية وفقًا لـ HWE (P <0.05) للمجموعتين. وجد تأثير اثنين من الأليلات على قابلية الإصابة بـ RA، وكان هناك ارتباط كبير بين CT60 و +49 مع خطر الاصابة بال RA، ويمكن أن يكون كل من CT60 و +49 السبب الرئيسي لـ RA، بينما JO31 وMH30 غير مرتبطين بـ CTLA–4.

الكلمات المفتاحية: RA،CT60 ، CTLA–4 ، +49، طرز وراثية

Received:14/12/2020, Accepted:22/3/2021

INTRODUCTION

Cytotoxic T Lymphocyte Associated protein 4 (CTLA4), is a well know protein receptor that regulates the responses of the immune system and that's why it is known "immune checkpoint" due to its role on some immunity pathways (18,41) . CTLA4 is expressed in activated T-lymphocyte and can be distinguished in cancer phenomena (23, 24). However, the variances in genes will increase the susceptibility and the possibility of diseases that are related to the autoimmune system and one of the main factors that's increase the risk in cancer the coding of the gene which may regulate the response of the immune system downward (8). In 2015, Walker and Sansom (36) showed that the effect of CTLA4 inhibition can be noticed by binding with B7 to form CTLA-4-B7 to suppress the antigen cells, and in 2017 Walker (37) again confirmed that the CTLA-4-B7 function is to bind with the secondary signal to activate the response of the immune system. In 2017 Lo (21) and Abdel-Mota, on a study on mice, they showed that decreasing the CTLA-4 amounts will cause fatal and autoimmunity, and this deficiency is associated with a lot of diseases such as; Rheumatoid arthritis (karemi references), type 1 diabetes (30) , Lupus (16) , Celiac disease (11), and a lot more. In this study, the research focus on the risk of increase of autoimmune diseases in particular Rheumatoid Arthritis (RA) (27) which a disease that is considered as a chronic, and rheumatic inflammation which ultimately cause destruction in the joint that's end with disability and complications (22 , 31) in the whole joint system which characterized by the physicians as autoimmune disease that could also be related to the interaction of the mammals with the environment as well as the polymorphisms in certain genes (genetic factors), and until now, and with the help Of GWAS technology, it has been characterized more than 100 gene were associated with RA (38). However, all these studies need more investigation (26). Understanding the risk factor of the gene and its association with RA pathogenesis, it is essential to study and investigate the pathology of RA from the standpoint of genomes role (2, 9, 12). The current study focus on a certain protein receptor CTAL4 and its association with RA due to its relation with, autoimmune, and its role in the immunity pathways such as the possibility of variation in code of some genomes.

MATERIALS AND METHODS

This study was carried out at Baghdad Teaching Hospital , Medical City , Baghdad , Iraq. In this study, 245 patients diagnosed with RA were selected in this study (177 females and 68 males) with average mean age around 46 (46.15) while the healthy individuals (control group) were 188 consist of 133 females and 55 males with average mean age around 45 (45.08) and they were selected randomly as volunteers from the same area, they were characterized by specialists that they do not have any health issues or any diseases that related to RA. The diagnosis of the RA to both patients group and healthy group (control group) were characterized according to the protocols depicted by Arnett et al., 1988 (3). The genotype sequences to certain genes were identified using PCR-DGGE, and the procedure was carried out according to the protocol explained by Ying Wu et al., 1998, and Vanessa et al., 1999 respectively(35 ,39). Table 1 shows the fragment of PCR-DGGE, the bp represent the based pair, while the T_m is the temperature that was annealed in the test.

Table1.PCR-DGGE primers to the detected SNP

Fragment	Primer 5'-3'	T_m (° C)	Size of fragment (bp)
JO31	[32GC] GTATCATCTCAATGGGTTGTTCC GCAGGCGGACAACACAAA	56 °C	≅ 149
CT60	[32GC] AAGTCATTCTTGGAAGGTAT CAACTGTAATGCCTGTGATA	50 °C	≅ 220
+49	[32GC] CCTGAAAGGTTTTGCTCTA AGAAGACAGGGATGAAGAG	51 °C	≅ 196
MH30	[32GC] AATGCTCAGTTTTATGACCCAA TGCCCATCAGCAGCCTAT	58 °C	≅ 175

The frequencies of allele and the genotype were considered and calculated for both control group and the patients group and the confirmation of HWE (Hardy-Weinberg Equilibrium) along with x^2 which was applied to determine the difference in the genotype between control and patients statistically, the analysis was carried out according to Tang et al., 2002 (32). The P-value was considered significant when its value is less than 0.05 (P<0.05).

RESULTS AND DISCUSSION

Four Single nucleotide polymorphisms (SNPs) were genotyped for the control and the patients 188 individuals, and 245 RA patients respectively, Table 2 shows the distribution of the genotypes was carried out according to HWE (P<0.05) for the two groups (control and RA patients).

Table 2. Distribution of the genotypes under investigation

	Control (188)	RA Patients (245)	P-value	X^2
JO31				
G/G	**77(40.9%)**	**112(45.7%)**		
T/T	**19(10.2%)**	**27(11.0%)**	**0.298**	**1.99**
G/T	**92(48.9%)**	**106(43.3%)**		
CT60				
G/G	**63(33.5)**	**119(48.5%)**		
A/A	**23(12.2%)**	**25(10.1%)**	**0.006**	**10.23**
G/A	**102(54.3%)**	**101(41.4%)**		
+49				
G/G	**61(32.5%)**	**113(46.1%)**		
A/A	**27(14.6%)**	**31(12.6%)**	**0.019**	**7.69**
G/A	**100(52.9%)**	**101(41.3%)**		
MH30				
G/G	**88(46.8%)**	**108(44.1%)**		
C/C	**14(7.4%)**	**31(12.6%)**	**0.098**	**3.95**
G/C	**86(45.8%)**	**106(43.3%)**		

The four SNPs and the frequencies of their genotypes (using HWE) were showed in table 2 for both control and patients groups, and it is very clear that JO31 and MH30 were not shown any significant differences between control group and RA patients group, on the other hand, CT60 and +49 genotypes shows a significant differences between control group and RA patients group (P<0.006, P<0.019 respectively), this indicates that these two genotypes (CT60 allele G and +49 allele) were related and associated to the increasing of RA risk. Table 3 shows the effect of these two alleles on the susceptibility of RA, and as it is clears, there is a major association between the CT60, +49 alleles with the risk of RA (OR 1.43, 95%Cl (1.14-1.89), and the *P*-value 0.004 for CT60), while for +49 it was (OR 1.38, 95%Cl (1.04-1.77) and the P-value 0.009).

Table 3. Effect of four alleles on the RA susceptibility

	Control	RA Patients	OR (95%Cl)	P-value	X^2
JO31					
G	**238(63.4)**	**325(66.5)**	**1.08(0.79-1.44)**	**0.317**	**1.08**
T	**137(36.6)**	**164(33.5)**	**0.88(0.67-1.148)**		
CT60					
G	**224(59.8)**	**342(69.9)**	**1.43(1.14-1.89)**	**0.004**	**8.92**
A	**151(40.2)**	**147(30.1)**	**0.73(0.58-.94)**		
+49					
G	**225(60.1)**	**332(67.9)**	**1.38(1.04-1.77)**	**0.009**	**6.71**
A	**150(39.9)**	**157(32.1)**	**0.75(0.55-0.96)**		
MH30					
G	**261(69.7)**	**321(65.6)**	**0.85(0.67-1.11)**	**0.225**	**1.28**
C	**114(30.3)**	**168(34.4)**	**1.19(0.92-1.53)**		

The haplotypes that possibly constructed by CT60 and +49 was also analyzed; also the frequencies of haplotype were predicted using HyPop program to reach the maximum expectation, the analysis shows that there is a disequilibrium association with two haplotypes sets of SNPs in the control individuals, while the association was with four in patient group. Table 4 shows the correlation between the CTLA-4:+49:CT60 estimated and the RA haplotypes.

Table 4. Correlation of predicted haplotypes of CTAL-4 +49:CT60 with RA

Haplotype	Control %	Patients %	OR (95%Cl)	X2	P-value
+49A: CT60A	109(29.06)	107(21.88)	---	---	---
+49G: CT60A	37(9.87)	47(9.61)	1.43(0.87-2.36)	2.81	0.0975
+49A: CT60G	42(11.21)	59(12.07)	1.55(1.01-2.42)	4.25	0.0416
+49G: CT60G	187(49.86)	276(56.44)	1.58(1.19-2.12)	10.16	0.0012

The haplotype of +49A: CT60G and +49G: CT60G have further frequency than +49G: CT60A, and +49A: CT60A, and by comparing the findings of these four haplotypes between control individuals and the patients, there is a significant difference in p-value, X^2, as well as in OR (95%Cl), ultimately, the calculated p-value was 0.0012 for +49G: CT60G, and it was 0.0416 for +49A: CT60G which indicates an important correlation between these haplotypes (CTAL-4 +49:CT60) with the RA. The significance of CTLA-4 molecule is its role on the production of IL-2 as well as on its receptors and the soluble CTLA-4 (sCTLA-4) which is allied with the diseases of the autoimmune system such as Systemic Sclerosis, Systemic Lupus Erythematosus, and Thyroid Disease (4 , 25). The findings of the current study showed that there is no important correlation between CTLA4 with JO31 , and MH30 with RA .On the other hand there is a susceptible effect and risk of RA with other two SNPs (CTLA-4 with CT60, and +49) (10 ,14). it is very important to mentioned that these findings are based on study on Iraqi population which cannot be applicable on other population due to the difference in genes. The polymorphism of CTLA-4-+49 was genotyped widely in many autoimmune diseases such as RA (20, 34) Behçet's Disease (1), pathogenesis of Graves' disease (19), Oral Lichen Planus disease (6), and systemic lupus erythematosus and its geographical distribution (17). The polymorphism of CTLA-4 and CT60 was also genotyped widely in a lot of autoimmune diseases suach as; RA (28, 33), Thalassemia (29), Sjögren Syndrome (7), Polymorphism in Renal Cell Cancer (5), Production of Thyroid Autoantibody (40) , Polycystic ovarian syndrome (13,15).

REFERENCES

1. Abdel Galil SM and HA Hagrass.2014. The Role of CTLA-4 Exon-1 49 A/G Polymorphism and Soluble CTLA-4 Protein Level in Egyptian Patients with Behçet's Disease. Biomed Research International. 2014: 513915. doi: 10.1155/2014/513915

2. AL-Hamdani H. and A AL-Hashimy.2020. Molecular detection of UREC, HPMA, RSBA AND MRPA genes of Proteus Mirabilis urinary tract infection in patient with rheumatoid arthritis. Iraqi Journal Of Agricultural Scineces. 51(Special Issue). 245-251

3. Arnett FC,SM Edworthy and DA Bloch.1988. The American rheumatism association 1987 revised criteria for the classification of the rheumatoid arthritis. Arthritis Rheum (31): 315- 324

4. Buchbinder EI and A Desai.2016. CTLA-4 and PD-1 Pathways. American Journal of Clinical Oncology. 39(1): 98-106.

5. Cozara JM, JM Romerob, N Aptsiaurib, F Vazqueza and et al.2007. High incidence of CTLA-4 AA (CT60) polymorphism in renal cell cancer. Human Immunology. 68: 698-704.

6. Ghapanchi J, MR Haghshenas, H Ghaderi, S Amanpour and et al.2014. CTLA-4 Gene Polymorphism in +49 A/G Position: A Case Control Study on Patients with Oral Lichen Planus. Journal of International Oral Health. 6(5): 17-21

7. Gottenberg J-E, P Loiseau, M Azarian, C Chen and et al.2007. CTLA-4 +49A/G and CT60 gene polymorphisms in primary Sjögren syndrome. Arthritis Research and Therapy. 9(2): R24.

8. Gough SC, LS Walker and DM Sansom.2005. CTLA4 gene polymorphism and autoimmunity. Immunology Reviews. (204): 102-115

9. GTEx Consortium. Human genomics.2015. The Genotype-Tissue Expression (GTEx) pilot analysis: multi tissue gene regulation in humans. Science. (348): 648–660

10. Hannani D, M Vétizou, D Enot, S Rusakiewicz and et al.2015. Anticancer immunotherapy by CTLA-4 blockade: obligatory contribution of IL-2 receptors and negative prognostic impact of soluble CD25. Cell Research. 25(2): 208–224

11. Hunt KA, DP McGovern, PJ Kumar, S Ghosh and et al.2005. A common CTLA4 haplotype associated with coeliac disease. European Journal of Human Genetics. 13(4): 440-4.

12. Ishigaki K, Y Kochi, A Suzuki and et al.2017. Polygenic burdens on cell-specific pathways underlie the risk of rheumatoid arthritis. Nature Genetics. (49): 1120-1125

13. Jasim R.A., M. A. Umran and E. H. Humadi. 2020. Detection of vaspin RS2236242 and chemerin RS17173608 gene polymorphism in a sample of obese Iraqi polycystic ovarian syndrome women. Iraqi Journal of Agricultural Sciences .51(1):462-469

14. Jia X-H, L-Y Geng, P-P Jiang, H Xu and et al. 2020. The biomarkers related to immune related adverse events caused by immune checkpoint inhibitors. Journal of Experimental & Clinical Cancer Research. (39): 284.

15. Jing Su, Li Yan , Su Guanglong ,J Wang, T Qiu.2018.Genetic association of CTLA4 gene with polycystic ovary syndrome in the Chinese Han population. Medicine. 97: 29(e11422).

16. Kailashiya V, HB Sharma , J Kailashiya.2019. Role of *CTLA4* A49G polymorphism in systemic lupus erythematosus and its geographical distribution. Journal of Clinical Pathology. 10: 659-662.

17. Kailashiya V, HB Sharma , J Kailashiya . 2019.Role of *CTLA4* A49G polymorphism in systemic lupus erythematosus and its geographical distribution. Journal of Clinical Pathology. 72**:** 659-662

18. Karami J, S Aslani, A Jamshidi, M Garshasbi and M Mahmoudi.2019. Genetic implications in the pathogenesis of rheumatoid arthritis; an updated review. Gene. (20)702:8-16.

19. Kouki T, Y Sawai, CA Gardine,ME Fisfalen ME .2000. CTLA-4 gene polymorphism at position 49 in exon 1 reduces the inhibitory function of CTLA-4 and contributes to the pathogenesis of Graves' disease. Journal of Immunology. 165(11): 6606-6611.

20. Lee CS, YJ Lee .and HF Liu .2003. Association of CTLA-4 gene A-G polymorphism with rheumatoid arthritis in Chinese. Clinical Rheumatology. (22): 221-224

21. Lo B and UM Abdel-Motal. 2017..Lessons from CTLA-4 deficiency and checkpoint inhibition. Curr Opin Immunol. (49): 14-19.

22. MacGregor AJ, H Snieder, AS Rigby and et al. 2000. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheumatic. (43): 30-37

23. Meggyes M, J Szanto, A Lajko, B Farkas and et al. 2018. The possible role of CD8+/Va7.2+/CD161++ T (MAIT) and CD8+/Va7.2+/CD161 lo T (MAIT-like) cells in the pathogenesis of early-onset pre-eclampsia. American Journal of Reproductive Immunology. 79:e12805.

24. Miko, E., M Meggyes, , K .Doba., A. Barakonyi,. and L. Szereday.2019. Immune Checkpoint Molecules in Reproductive Immunology. Frontiers in immunology. 10: 846.

25. Oaks MK, KM Hallett ,RT Penwell and et al.2000. A native soluble form of CTLA-4. Cell Immunology. (201): 144- 153

26. Okada Y, D Wu, G Trynka and et al.2014. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature. (506): 376-381

27. Okada Y, S Eyre ,A Suzuki , Y Kochi and et al. 2019.Genetics of rheumatoid arthritis: 2018 status. Annals of the Rheumatic Diseases. Annals of Rheumatic Diseases. 78(4)**:** 446-453

28. Orozco G, B Torres, A Núñez-Roldán, MF González-Escribano and J Martín.2004. Cytotoxic T-lymphocyte antigen-4-CT60 polymorphism in rheumatoid arthritis. Tissue Antigens. 64(6): 667-670.

29. OrrÚ S, N OrrÚ, E. Manolakos, R. Littera and et al.2012. Recipient CTLA-4*CT60-AA genotype is a prognostic factor for acute graft-versus-host disease in hematopoietic stem cell transplantation for thalassemia. Human Immunology. 73: 282-286

30. Saleh HM, N. Rohowsky and M. Leski.2008. The CTLA4 -819 C/T and +49 A/G dimorphisms are associated with Type 1 diabetes in Egyptian children. Indian J Human Genetics. 14(3): 92-98. doi: 10.4103/0971-6866.45001

31. Smolen JS, D. Aletaha, A. Barton and et al.2018. Rheumatoid arthritis. Natural Reviews in Diseases Primers 4: 18001

32. Tang G, H. Xie, L. Xu, Y. Hao, D. Lin and D. Ren.2002. Genetic study of apolipoprotein E gene, alpha-1 antichymotrypsin gene in sporadic parkinson disease. Am J Med Genet. (114): 446- 449.

33. Tsukahara S, T. Iwamoto, K. Ikari and et al.2008. CTLA-4 CT60 polymorphism is not an independent genetic risk marker of rheumatoid arthritis in a Japanese population. Annals of the Rheumatic Diseases. 67**:** 428-429

34. Vaidya B, SH. Pearce, S. Charlton and et al.2002. An association between the CTLA-4 exon 1 polymorphism and early rheumatoid arthritis with autoimmune endocrinopathies. Rheumatology (Oxford) (41): 1213

35. Vanessa MH, W.Ying, O Jan and et al. 1999. Improvements in gel composition and electrophoretic conditions for broad-range mutation analysis by denaturing gradient gel electrophoresis. Nucleic Acids Research. 27: e29

36. Walker LSK and DM Sansom. 2015. Confusing signals: recent progress in CTLA-4 biology. Trends in Immunology. (36): 63-70.

37. Walker LSK. 2017. EFIS lecture understanding the CTLA-4 checkpoint in the maintenance of immune homeostasis. Immunology Letters. (184): 43-50.

38. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007; 447: 661-678

39. Ying Wu, VW Hayes, Osinga Jan and et al.1998. Improvement of fragment and primer selection for mutation detection by denaturing gradient gel electrophoresis. Nucleic acids Res. (26): 5432-5440

40. Zaletel K, B. Krhin, S. Gaberscek, A. Bicek A, T Pajic, S. Hojker.2010. Association of CT60 cytotoxic T lymphocyte antigen-4 gene polymorphism with thyroid autoantibody production in patients with Hashimoto's and postpartum thyroiditis. Clin Exp Immunol. 1;161(1):41-7.

41. Zhou C, S. Gao, Z. Shu, S. Li and et al. 2020. Association between CTLA-4 Gene Polymorphism and Risk of Rheumatoid Arthritis (RA): Evidence from a Meta-analysis Involving 21681 Cases and 23457 Controls.