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Association of *CTLA-4* (+49A/G) polymorphism and susceptibility of developing rheumatoid arthritis in an Iraqi Arab population

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ABSTRACT

Background: The gene responsible for encoding the protein of cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) has been found to be associated with rheumatoid arthritis (RA) in different ethnic populations. But the association of +49A/G CTLA-4 polymorphism with susceptibility of RA among Iraqi Arab populations has not yet been determined.

Methods: One hundred and seventy-eight patients were examined, 67 of them were males (mean age 54.71 ± 10.4 years), while 167 were examined for the control group, of whom 64 were males and the rest were females. *CTLA-4* DNA genotyping was carried on to determine the +49 A/G (rs231775) polymorphism using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Enzyme-linked immunosorbent assay (ELISA) was also applied here to measure the antibodies level for cyclic citrullinated peptides (anti-CCP) and Rheumatoid factor (RF).

Results: The frequency of AG and GG genotypes in *CTLA-4* + 49 were significantly higher among RA patients in comparing with controls (55.61% vs 42.51%, OR = 2.18, 95% CI = 1.62–3.79, $P = 0.003$) and (20.22% vs 10.77%, OR = 2.61, 95% CI = 1.31–6.46, $P = 0.002$) respectively. G allele frequency was also significantly higher among RA cases (52.24% vs. 31.73%, OR = 3.02; 95% CI = 1.61–7.39, $P = 0.001$). The frequencies of the AA genotype and A allele, however, were significantly lower in cases than controls (24.15% vs 46.70%, $P = 0.001$) and (47.75% vs 68.26%, P = 0.001) respectively. Moreover, the levels of Anti-CCP and RF were raised significantly among RA patients than controls ($P = 0.0001$), but none of these parameters were correlated with genotypes of *CTLA-4*.

Conclusions: Carries of *CTLA-4* + 49 AG and GG alleles were at a high risk of developing functional disability of RA, unlike the AA allele carriers.

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disorder that has been reported to affect approximately 1% of adults in all populations. Destruction of synovial joints and persistent inflammation, which most likely leads to subsequent changes in joint integrity, are common symptoms of RA (McInnes and Schett, 2017). Furthermore, among the characteristics of rheumatoid arthritis are the increased levels of autoantibodies, the destruction of bone and cartilage that leads to fatigue and chronic pain, and subsequently permanent disability can occur as a result of persistent osteoarthritis and synovitis (Wang et al., 2014). The etiology of RA could be triggered due to the involvement of complex networks of immune elements such as T-cells, B-cells, plasma cells, mast cells, and dendritic cells, and cytokines (pro-inflammatory and anti-inflammatory) (Siebert et al., 2015). Although the genetic and environmental factors are believed to hold a crucial role in the occurrence of this disorder, the precise etiology is still uncertain. Clinical and genetic studies have revealed that genetic disorders are among the important risk factors and the main determinants of RA susceptibility (Goëb et al., 2008; Korhonen and Moilanen, 2009). It has been shown that RA has a genetic basis, the human leukocyte antigen (HLA) class II genes have been identified as the most powerful genetic indicators so far, that could be responsible for counting 50% of familial RA and 20% of all RA cases (Hasstedt et al., 1994). Furthermore, this correlation accounts for only about one-third of genetic susceptibility, and non-HLA genes, such as those that are important in the regulation of T-cell response, which are

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assumed to be a risk factor of RA (Costenbader et al., 2008).

The Cytotoxic T lymphocyte antigen-associated 4 (*CTLA-4*) gene has been identified as a susceptible gene correlated with autoimmunity diseases (Romo-Tena et al., 2013). *CTLA-4*, which is located on the 2q33 chromosome, acts as a negative regulator of T-cell activation. *CTLA-4* has a leather peptide sequence encoded by the the 5′ gene region of the first exon, in addition to the three other exons that encode an extracellular V-like domain (an immunoglobulin domain), a transmembrane domain, and a cytoplasmic tail domain respectively. The *CTLA-4* gene encodes a 223 amino acid precursor polypeptide, the mature transmembrane (TM) protein is produced after cleaving of 37 amino acids of the precursor, a process that is positively controlled by T-cell activation (Chan et al., 2014). CTLA-4 and CD28 are homologs; CTLA-4 controls T-cell proliferation and is critical for IL-2 accumulation; it also mediates in T-cell apoptosis via its binding with B7 molecules on antigen-presenting cells. The B7/CD28-CTLA4 complex involve in a co-stimulatory pathway of T-cell activation. According to that, it is believed that the CTLA4 gene is a significant candidate for autoimmune disease susceptibility (Yokosuka et al., 2010). Numerous T-cell-mediated autoimmune disorders have been correlated with *CTLA-4* polymorphisms, including type 1 diabetes (T1D), multiple sclerosis, and Graves' disease (Marron et al., 2000; Kantarci et al., 2003; Ramgopal et al., 2018). However, there are some controversial reports regarding the correlation of *CTLA-4* and RA (Plenge et al., 2005; Sfar et al., 2010; Tang and Zhou, 2013).

The correlation between the *CTLA-4* polymorphism and clinical characteristics of RA has not yet been fully clarified (Tsukahara et al., 2008; Muñoz-Valle et al., 2010a). The +49A/G polymorphism (rs231775) located in exon 1 of the *CTLA-4* gene has been poorly reported to be possibly related to RA as a genetic risk factor. The non-synonymous substitution in the 17 amino acids caused by rs231775 exhibits an inhibitory effect on *CTLA-4* function via reducing cell surface expression (Tang and Zhou, 2013; Elshazli et al., 2015; Fattah et al., 2017; Aslam et al., 2020). Although the association between the occurrence of RA among carriers of rs231775 has been studied before, the data are still limited and inconclusive (Lei et al., 2005; Benhatchi et al., 2011; Li et al., 2014; Zhou et al., 2021). Anti-CCP and RF antibodies hold promise for the early and more accurate diagnosis of RA, provide greater prognostic information, and have been implicated in RA pathogenesis (Huang et al., 2020). Here, we have conducted a similar study to define the association between RA and SNP of rs231775, which has not been performed before among Iraqi Arab RA patients. The *CTLA-4* + 49A/G polymorphism and the serum levels of RF and Anti-CCP have been investigated.

2. Material & methods

2.1. Study subjects

A total of 178 RA patients (67 males and 111 females) with mean age (54.71 ± 10.4 years) were voluntarily enrolled for this study. Patients were diagnosed in the consultant clinic of the Orthopedic and Bone Diseases department at Al-Sader hospital in Al-Najaf city between July 2019 and February 2020. Physicians confirmed the diagnosis based on symptoms and laboratory tests that revealed elevated RF and Anti-CCP levels in serum. Patients with any underlying disease except RA, such as those who had suffered from kidney diseases, pregnancy and lactation, autoimmune disorders, hepatic functions, and history of malignancy were excluded. The enrolled patients had not previously been on long-term medication. Controls were 167 (64 males and 103 females) with mean age (52.53 ± 10.1 years), who were clinically healthy individuals and randomly chosen during the period of samples collection. Subjects of control were free of any clinical evidence of autoimmune diseases or familial history of RA with a low level of serum RF and Anti-CCP.

2.2. Methods

2.2.1. Samples collection

Five milliliters of whole blood were collected from individuals shortly after diagnosis. For DNA extraction, 3 ml of the sample was frozen, while the rest was processed to separate serum, which was subsequently used for ELISA assay (Omega Diagnostics kit) to measure RF and anti-CCP levels.

2.2.2. DNA extraction and + 49A/G CTLA-4 genotyping

Blood genomic DNA extraction was carried out according to the protocol of "DNA purification from blood or body fluids using the spin protocol" mentioned in QIAamp DNA Mini and Blood Mini Handbook of Qiagen. NCBI database was used to define the *CTLA-4* SNP of rs231775. The PCR–RFLP assay was applied here for genotyping determination. PCR was used to amplify a 162 bp product using specified primers designed by Hajilooi et al., 2014, the primers' sequences have been checked based on the information of the gene sequence deposited in NCBI (RefSeq NG_011502.1, Accession version; NG_011502.1). PCR mixture was set according to the manufacturer (GenetBio, Korea) with the following program, 95 °C for ten minutes, followed by 30 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 40 s, using the following primers: 5′GCTCTACTTCCTGAAGACCT3′ and 5′AGTCTCACTCAC CTTTGCAG3′. The PCR product was then cleaved with the *BbvI* restriction enzyme (Fermentas). To permit the distinction of the restriction pattern, the digested product was loaded on an agarose gel stained with GelRed. By cleaving the amplified DNA, the presence of restriction sites indicating the existence of the G allele. The presence of a 162 bp single band indicates that the genotype of the subject is AA, whereas the GG genotype was determined when two bands of 91 bp and 71 bp appeared. Furthermore, the presence of three bands (162 bp, 91 bp, and 71 bp) identified the AG genotype, as shown in Table 1.

2.2.3. Rheumatoid factor (RF) and anti-CCP measurements

The serum levels of RF and anti-CCP were measured in samples of the study subjects using an ELISA kit (Omega Diagnostics, UK). The microwell plate, set of reagents, and patients' sera were left to reach room temperature for thirty minutes. Serum samples were diluted by sample buffer at 1:101. Each plate contains anti-CCP standards, positive and negative controls, and dilutions of standards and controls. Horseradish peroxidase-conjugated anti-human immunoglobulins were used to treat microliter plates, and antigen-antibody complexes reacted with them. An enzymatic (blue) colorimetric reaction was occurred after applying three washes, the plates were read at 450 nm after 30 min of being added to the TMB substrate and diluted acid (color change to yellow). When the absorbance value exceeded the kit's cut-off (18 U/ml), anti-CCP antibodies were considered positive. The amount of conjugate bound to the antigen-antibody complex determines the rate at which color is formed from the chromogen, and this is inversely proportional to the initial concentration of antibodies in the patient sample. RF principle is similar to that principle of Anti-CCP. As long as the IgM-RF level was less than 20 U/ml and greater than 20 U/ml, this level was considered normal. Utilizing Plasmatic Laboratory Products, the

Table 1
Clinical characteristics of RA patients and control groups.

Variable	Control ($n = 167$)		RA patients ($n = 178$)		*P*-Value
	No.	(%)	No.	(%)	
Sex:					
Male	64	(38.32%)	67	(37.32%)	0.68
Female	103	(61.68%)	111	(62.36%)	
Age (mean ± SD)	52.53 ± 10.1		54.71 ± 10.4		0.31
Anti-CCP (mean ± SD)U/ml	8.58± 5.17		79.14 ± 59.52		0.000*
RF(mean ± SD)U/ml	10.19 ± 5.83		234.61 ± 47.64		0.000*

manufacturer's instructions for immunoagglutination tests were followed for the qualitative and semi-quantitative assessment of RF. A linear regression equation on known concentration standards was used to calculate RF and Anti-CCP concentrations in serum samples.

2.3. Statistical analysis

A statistical package of the social science (SPSS) version 20 software was used for data analysis of molecular and biochemical factors. The values were expressed as mean ± SD for each variable. One-Way Analysis of Variance (ANOVA) was carried on for data evaluation which was followed by Tukey's multiple comparison test. The relative risk was assessed with rare alleles using an odds ratio (OR) with a 95% confidence interval (CI), "P" value was regarded as significant when it was less than 0.05.

3. Results

As shown in Table 1, there were no statistically significant differences between the two studied groups in age and sex ($P = 0.68$ and 0.31, respectively). Anti-CCP and RF levels were significantly higher in RA patients than controls (79.14 ± 59.52 vs. 8.58± 5.17), (234.61 ± 47.64 vs. 10.19 ± 5.83) ($P = 0.0001$).

PCR products of *CTLA-4* (+49A/G) SNP in RA patients and controls were subjected for restriction digestion with *BbvI* to characterize the genotyping of the study subjects (Fig. 1). The genotyping and allele frequencies determination of the *CTLA-4* (+49 A/G) SNP are as revealed in Table 2. The frequency of the AG and GG genotypes in RA cases were statistically higher significant in comparison to the control group, [55.61%, OR = 1.69, 95% CI = 1.10–2.59, $P = 0.01$] and [20.22%, OR = 2.09, 95% CI = 1.13–3.86, $P = 0.01$] respectively. Regarding the allelic distribution, G allele were higher in cases than its distribution in controls (48.04% vs. 30.04%, OR = 1.96; 95% CI = 1.43–2.67, $P = 0.0001$).

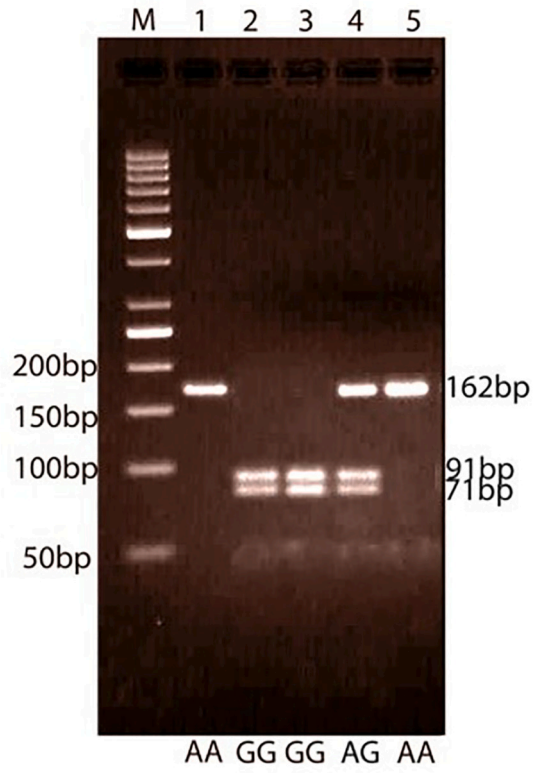

Fig. 1. PCR products of +49A/G CTLA-4 polymorphism after restriction digestion with *BbvI* (2%) agarose gel electrophoresis. Lane (M) is a 100 bp DNA Ladder. Lane (1,5) AA genotype 162 bp, Lane (2,3) GG genotype 91 and 71 bp, Lane (4) AG genotype 162, 91 and 71 bp.

Regarding the correlation of +49A/G *CTLA-4* genotypes and the sex of the study subjects, results indicate that there was no significant correlation between AA, AG, and GG genotypes and gender of RA patients ($P = 0.42$, 0.39, and 0.45) respectively (Table 3). Additionally, as indicated in Table 4, there was no significant association between levels of anti-CCP and RF and the AA, AG, or GG genotypes ($P = 0.33$ and 0.25), respectively.

4. Discussion

The importance of CTLA4 + 49A/G polymorphism in susceptibility to RA in Arab ethnicity of the population of Iraq has not been revealed before. Results of the current study, which was conducted for the first time in the middle Euphrates region of Iraq, reveals that the A/G, G/G, and G alleles of CTLA4 were associated with a higher incidence of RA. The A/A genotype and A allele, however, were found to be less prevalent among RA patients, it is highly likely correlated with protection against RA. In accordance with the present results, previous studies have demonstrated the same finding among different ethnical populations especially in Asians (Zhou et al., 2021), Chinese (Tang and Zhou, 2013; Li et al., 2014), Japanese (Yanagawa et al., 2000), Pakistani (Sameem et al., 2015; Aslam et al., 2020), and among Caucasians in British (Vaidya et al., 2002), Hungarian (Magyari et al., 2007), Dutch (Van Belzen et al., 2004), in addition to the Africans ethnicity such as Egyptian (Elshazli et al., 2015; Fattah et al., 2017), and Mexicans population (Muñoz-Valle et al., 2010a).

This result could be explained by a + 49A/G transition polymorphism in exon1's leader sequence, which leads to the substitution of Thr for Ala at amino acid position 17. which probably changes the biochemical characteristics of the protein and eventually affects its function. The hydrophobic alanine is added into the CTLA4 leader peptide, this modification might impair the intracellular transport of the CTLA4 protein and subsequently leads to reduce its activity on the cell surface. Due to the reduction in the number of functionally active CTLA4 molecules on the T lymphocytes' cell surface among carriers of GG homozygotes, fewer B7-CTLA4 complexes may form, resulting in CTLA4-mediated T cell suppression which is less effective. As a result, a considerable increase in T cell proliferation can be detected when compared to AA homozygotes (Mäurer et al., 2002; Anjos and Polychronakos, 2004). Furthermore, reduced CTLA-4 expression may stimulate the proliferation of T cells which predispose them to autoimmune disorders like RA. Moreover, the amino acid substitution due to the +49A/G SNP was thought to be correlated with malfunctioning the endoplasmic reticulum processing of a substantial number of CTLA4 molecules, leading to decreasing cell surface expression and abnormally glycosylated product, this causes a reduction in both T-cell proliferation control and inhibitory activity of CTLA4. Thus, the mutant G allele is possibly an important risk factor of RA (Ueda et al., 2003; Park et al., 2007).

Contradictory results were reported which revealed that no association was found between genotypes and alleles frequency of *CTLA-4* + 49 A/G and the occurrence of RA, in the same populations aforementioned such as Asians like Chinese (Liu et al., 2013), Koreans (Kim et al., 2010), and Caucasians such as Poland's (Luterek-Puszyńska et al., 2017) and Slovaks (Benhatchi et al., 2011), in addition to other ethnicity populations such as Africans-Americans (Kelley et al., 2009) and Africans-Tunisians (Sfar et al., 2010). The inconsistency of results among different populations enlightens the role of genetic background in the development of RA in various ethnic populations may be attributable to the fact that these populations have different allele frequencies for this polymorphism. For example, the G allele frequency in Caucasians was relatively low (37.5%), comparing to that in Asians (62.7%) (Lee et al., 2012). Other factors such as the exposure to different environmental risk factors in each particular population, in addition to the

Table 2
Genotyping and allele frequencies of +49A/G CTLA-4 SNP.

GENOTYPE ALLELE	CONTROL (*N* = 167)		RA (N = 178)		OR	95%CI	P-VALUE
	%	No.	%	No.			
Codominant							
AA	46.7	78	24.15	43	Reference		
AG	42.51	71	55.61	99	1.69	1.10–2.59	
GG	10.77	18	20.22	36	2.09	1.13–3.86	**0.01***
Dominant							
GG	10.77	18	20.22	36	Reference		
AA+AG	89.22	149	79.77	142	2.3	0.25–0.87	**0.01***
Recessive							
GG + AG	53.29	89	85.95	135	Reference		
AA	46.7	78	24.15	43	0.36	0.22–0.57	**0.000***
Alleles							
A	67.96	227	51.96	185	Reference		
G	30.04	107	48.04	171	1.96	1.43–2.67	**0.000***

Table 3
Association between sex of RA patients with genotypes of +49A/G CTLA-4.

Genotype	Male (*n* = 67)	Female (*n* = 111)	P-Value
	% No.	% No.	
AA	56.71 38	42.34 47	**0.42**
AG	43.28 29	56.75 63	**0.39**
GG	41.79 28	58.55 56	**0.45**

Table 4
Correlation between Serum anti-CCP, RF, and + 49A/G CTLA-4 polymorphism in RA patients.

Clinical characteristic	Genotype Mean ± SD			*P*-Value
	AA(*n* = 43)	AG(*n* = 99)	GG(*n* = 36)	
Anti-CCP (mean ± SD) U/ml	80.32 ± 49.7	68.95 ± 57.36	75.44± 60.85	**0.33**
RF(mean ± SD) U/ml	242.38 ± 34.86	213.55 ± 58.11	224.79 ± 53.94	**0.25**

problems raised from the nature of research methodologies such as the sufficiency of sample size and use of a single technique for SNP detection, may be responsible for this controversial data (Elshazli et al., 2015). The gender of RA patients was not correlated with AA, AG, and GG genotypes in our study, which is consistent with two other studies that indicate no correlation between the gender of RA patients and + 49A/G and *CTLA-4* polymorphisms in codon 15 of exon 1 (Lee et al., 2003; Barton et al., 2004).

High levels of anti-CCP and RF was detected in RA patients compared with controls were observed in the current study. These results were in agreement with those obtained by (Sharif et al., 2007; Miriovsky et al., 2010). In RA cases, RF immunoglobulins (Ig) react specifically with the Fc fragment of the IgG molecule to form immune complexes as a kind of immune response against the disease (Syed et al., 2008). While, anti-CCP has an amino acids sequence known as the shared epitope, which is encoded a specific antigen called human leukocyte antigens (HLA) that is responsible for controlling the immune responses in RA patients (Bos et al., 2008). Furthermore, this study confirmed that G/G, A/G, AA genotypes in a leader of exon 1 of CTLA gene was not correlated with levels of anti-CCP and RF. Several studies were in accordance with our data and have demonstrated the lack of association between genotypes of +94 *CTLA-4* and anti-CCP or RF in RA patients. The reason behind the lack of the association perhaps due to the mutation of +49A/G which is located in codon 17 of exon 1, that is not specifically related to change the RF and anti-CCP levels, and perhaps there are other genetic variations responsible for the disease occurrence (John et al., 2005; Farago et al., 2007). Our results suggest that the *CTLA-4* exon-1 + 49G allele is highly likely to be a risk factor of RA among the Arab ethnicity of the population of Iraq.

Authors' contributions

K.S.Z.: Conducted the laboratory work, writing the first manuscript, designing of experiments, and analysis of results. B.K·K: designing the experiments, analyzing results, and writing the final manuscript. I.J.L: designing the experiments and analysis of results.

Declaration of Competing Interest

The authors declare that no conflicting interests exist.

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Ethical approval

All procedures used in studies involving human participants conformed to the institutional and/or national research committee's ethical requirements, as well as to the 1964 Helsinki declaration and its subsequent amendments or a comparable ethical standard. Also, all the participants obtained written informed consent.

References

Anjos, S., Polychronakos, C., 2004. Mechanisms of genetic susceptibility to type I diabetes: beyond HLA. Mol. Genet. Metab. 81 (3), 187–195.
Aslam, M.M., Jalil, F., John, P., Fan, K.H., Bhatti, A., Feingold, E., Kamboh, M.I., 2020. A sequencing study of CTLA4 in Pakistani rheumatoid arthritis cases. PLoS One 15 (9), e0239426.
Barton, A., Jury, F., Eyre, S., Bowes, J., Hinks, A., Ward, D., Worthington, J., 2004. Haplotype analysis in simplex families and novel analytic approaches in a case–control cohort reveal no evidence of association of the CTLA-4 gene with rheumatoid arthritis. Arthr. Rheum. 50 (3), 748–752.
Benhatchi, K., Jochmanová, I., Habalová, V., Wagnerová, H., Lazúrová, I., 2011. CTLA4 exon1 A49G polymorphism in Slovak patients with rheumatoid arthritis and Hashimoto thyroiditis—results and the review of the literature. Clin. Rheumatol. 30 (10), 1319–1324.
Bos, W.H., Bartelds, G.H., Wolbink, G.J., de Koning, M.H., van de Stadt, R.J., van Schaardenburg, D., Dijkmans, B.A., Nurmohamed, M.T., 2008. Differential response of the rheumatoid factor and anticitrullinated protein antibodies during adalimumab treatment in patients with rheumatoid arthritis. J. Rheumatol. 35, 1972–1977.
Chan, D.V., Ginson, H.M., Aufiero, B.M., Wilson, A.J., Hafner, M.S., Mi, Q.S., Wong, H.K., 2014. Differential CTLA-4 expression in human CD4+ versus CD8+ T cells is

associated with increased NFAT1 and inhibition of CD4+ proliferation. Genes Immunol. 15, 25–32.
Costenbader, K.H., Chang, S.C., Laden, F., Puett, R., Karlson, E.W., 2008. Geographic variation in rheumatoid arthritis incidence among women in the United States. Arch. Intern. Med. 168 (15), 1664–1670.
Elshazli, R., Settin, A., Salama, A., 2015. Cytotoxic T lymphocyte associated antigen-4 (CTLA-4)+ 49 A> G gene polymorphism in Egyptian cases with rheumatoid arthritis. Gene 558 (1), 103–107.
Farago, B., Talian, G.C., Maasz, A., Magyari, L., Horvatovich, K., Kovács, B., Melegh, B., 2007. Prevalence of functional haplotypes of the peptidylarginine deiminase citrullinating enzyme gene in patients with rheumatoid arthritis: no influence of the presence of anti-citrullinated peptide antibodies. Clin. Exp. Rheumatol. 25 (4), 523.
Fattah, S.A., Ghattas, M.H., Saleh, S.M., Abo-Elmatty, D.M., 2017. Cytotoxic T-lymphocyte-associated protein 4 gene polymorphism is related to rheumatoid arthritis in Egyptian population. Arch. Physiol. Biochem. 123 (1), 50–53.
Goëb, V., Dieudé, P., Daveau, R., Thomas-L'otellier, M., Jouen, F., Hau, F., Boumier, P., Tron, F., Gilbert, D., Fardellone, P., Cornélis, F., Le Loët, X., Vittecoq, O., 2008. Contribution of PTPN22 1858T, TNFRII 196R and HLA-shared epitope alleles with rheumatoid factor and anti-citrullinated protein antibodies to very early rheumatoid arthritis diagnosis. Rheumatol. (Oxford) 47 (8), 1208–1212.
Hasstedt, S.J., Clegg, D.O., Ingles, L., Ward, R.H., 1994. HLA-linked rheumatoid arthritis. Am. J. Hum. Genet. 55 (4), 738.
Huang, J., Zeng, T., Zhang, X., Tian, Y., Wu, Y., Yu, J., Tan, L., 2020. Clinical diagnostic significance of 14-3-3η protein, high-mobility group box-1, anti-cyclic citrullinated peptide antibodies, anti-mutated citrullinated vimentin antibodies and rheumatoid factor in rheumatoid arthritis. Br. J. Biomed. Sci. 77 (1), 19–23.
John, S., Davies, N., Worthington, J., 2005. Genes for rheumatoid arthritis offer new insights into disease mechanisms. Drug Discovery Today: Disease Mechanisms 2 (3), 337–344.
Kantarci, O.H., Hebrink, D.D., Achenbach, S.J., Atkinson, E.J., Waliszewska, A., Buckle, G., Weinshenker, B.G., 2003. CTLA4 is associated with susceptibility to multiple sclerosis. J. Neuroimmunol. 134 (1–2), 133–141.
Kelley, J.M., Hughes, L.B., Faggard, J.D., Danila, M.I., Crawford, M.H., Edberg, Y., Padilla, M.A., Tiwari, H.K., Westfall, A.O., Alarcón, G.S., Conn, D.L., Jonas, B.L., Callahan, L.F., Smith, E.A., Brasington, R.D., Allison, D.B., Kimberly, R.P., Moreland, L.W., Edberg, J.C., Bridges Jr., S.L., 2009. An African ancestry-specific allele of CTLA4 confers protection against rheumatoid arthritis in African Americans. PLoS Genet. 5 (3), e1000424.
Kim, Y.O., Kim, H.J., Kim, S.K., Chung, J.H., Hong, S.J., 2010. Association of the CD28/CTLA4/ ICOS polymorphisms with susceptibility to rheumatoid arthritis. Clin. Chem. Lab. Med. 48 (3), 345–353.
Korhonen, R., Moilanen, E., 2009. Abatacept, a novel CD80/ 86-CD28 T cell co-stimulation modulator, in the treatment of rheumatoid arthritis. Basic & Clinical Pharmacol. & Toxicol. 104 (4), 276–284.
Lee, C.S., Lee, Y.J., Liu, H.F., Su, C.H., Chang, S.C., Wang, B.R., Liu, T.L., 2003. Association of CTLA4 gene AG polymorphism with rheumatoid arthritis in Chinese. Clin. Rheumatol. 22 (3), 221–224.
Lee, Y.H., Bae, S.C., Choi, S.J., Ji, J.D., Song, G.G., 2012. Association between the CTLA-4+ 49 a/G polymorphism and susceptibility to rheumatoid arthritis: a meta-analysis. Mol. Biol. Rep. 39 (5), 5599–5605.
Lei, C., Dongqing, Z., Yeqing, S., Oaks, M.K., Lishan, C., Jianzhong, J., Daming, R., 2005. Association of the CTLA-4 gene with rheumatoid arthritis in Chinese Han population. Eur. J. Hum. Genet. 13 (7), 823–828.
Li, G., Shi, F., Liu, J., Li, Y., 2014. The effect of CTLA-4 A49G polymorphism on rheumatoid arthritis risk: a meta-analysis. Diagn. Pathol. 9 (1), 157.
Liu, C.P., Jiang, J.A., Wang, T., Liu, X.M., Gao, L., Zhu, R.R., Shen, Y., Wu, M., Xu, T., Zhang, X.G., 2013. CTLA-4 and CD86 genetic variants and haplotypes in patients with rheumatoid arthritis in southeastern China. Genet. Mol. Res. 12 (2), 1373–1382.
Luterek-Puszyńska, K., Malinowski, D., Paradowska-Gorycka, A., Safranow, K., Pawlik, A., 2017. CD28, CTLA-4 and CCL5 gene polymorphisms in patients with rheumatoid arthritis. Clin. Rheumatol. 36 (5), 1129–1135.
Magyari, L., Faragó, B., Bene, J., Horvatovich, K., Lakner, L., Varga, M., Melegh, B., 2007. No association of the cytotoxic T-lymphocyte associated gene CTLA4+ 49A/G polymorphisms with Crohn's disease and ulcerative colitis in Hungarian population samples. World J Gastroenterol: WJG 13 (15), 2205.
Marron, M.P., Zeidler, A., Raffel, L.J., Eckenrode, S.E., Yang, J.J., Hopkins, D.I., She, J.X., 2000. Genetic and physical mapping of a type 1 diabetes susceptibility gene (IDDM12) to a 100-kb phagemid artificial chromosome clone containing D2S72-CTLA4-D2S105 on chromosome 2q33. Diabetes 49 (3), 492–499.
Mäurer, M., Loserth, S., Kolb-Mäurer, A., Ponath, A., Wiese, S., Kruse, N., Rieckmann, P., 2002. A polymorphism in the human cytotoxic T-lymphocyte antigen 4 (CTLA4) gene (exon 1+49) alters T-cell activation. Immunogenet. 54 (1), 1–8.
McInnes, I.B., Schett, G., 2017. Pathogenetic insights from the treatment of rheumatoid arthritis. Lancet 389 (10086), 2328–2337.
Miriovsky, B.J., Michaud, K., Thiele, G.M., O'Dell, J.R., Cannon, G.W., Kerr, G., Richards, S., Johnson, D., Caplan, L., Reimold, A., Hooker, R., Mikuls, T., 2010. Anti-CCP antibody and rheumatoid factor concentrations predict greater disease activity in men with rheumatoid arthritis. Ann. Rheum. Dis. 69 (7), 1292–1297.
Muñoz-Valle, J.F., Valle, Y., Padilla-Gutiérrez, J.R., Parra-Rojas, I., Rangel-Villalobos, H., del Mercado, M.V., Ledezma-Lozano, I.Y., Villafan-Bernal, J.R., Armendáriz-Borunda, J., Pereira-Suárez, A.L., 2010a. The+ 49A> G CTLA-4 polymorphism is associated with rheumatoid arthritis in Mexican population. Clin. Chim. Acta 411 (9–10), 725–728.
Park, J.H., Chang, H.S., Park, C.S., Jang, A.S., Park, B.L., Rhim, T.Y., Uh, S.T., Kim, Y.H., Chung, I.Y., Shin, H.D., 2007. Association analysis of CD40 polymorphisms with asthma and the level of serum total IgE. Am. J. Respir. Crit. Care Med. 175 (8), 775–782.
Plenge, R.M., Padyukov, L., Remmers, E.F., Purcell, S., Lee, A.T., Karlson, E.W., Rioux, J. D., 2005. Replication of putative candidate-gene associations with rheumatoid arthritis in> 4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. Am. J. Hum. Genet. 77 (6), 1044–1060.
Ramgopal, S., Rathika, C., Padma, M.R., Murali, V., Arun, K., Kamaludeen, M.N., Balakrishnan, K., 2018. Interaction of HLA-DRB1* alleles and CTLA4 (+ 49 AG) gene polymorphism in autoimmune thyroid disease. Gene 642, 430–438.
Romo-Tena, J., Gómez-Martín, D., Alcocer-Varela, J., 2013. CTLA-4 and autoimmunity: new insights into the dual regulator of tolerance. Autoimmunity Rev. 12 (12), 1171–1176.
Sameem, M., Rani, A., Bashir, R., Riaz, N., Batool, S.A., Irfan, S., Nawaz, S.K., 2015. Ctla-4+ 49 polymorphism and susceptibility to rheumatoid arthritis in pakistani population. Pakistan J. Zool. 47 (6), 1731–1737.
Sfar, I., Dhaouadi, T., Krichen, H., Elbeldi, A., Abdelmoula, L., Makhlouf, M., Ayed, K., 2010. Genotyping CTLA-4 exon 1 (+49 A/G) and HLA-DRB1 polymorphisms and susceptibility to systemic lupus and rheumatoid arthritis in Tunisian population. J. Med. Genet. Genom. 2 (1), 008–017.
Sharif, S.K., Eghbal, S., Gharibdoost, F., Kbarian, M.A., Shahram, F., Nadji, A., Jamshidi, A.R., Davatchi, F., 2007. Comparative study of anti-CCP and RF for the diagnosis of rheumatoid arthritis. APLAR J. Rheumatol. 10 (2), 121–124.
Siebert, S., Tsoukas, A., Robertson, J., McInnes, I., 2015. Cytokines as therapeutic targets in rheumatoid arthritis and other inflammatory diseases. Pharmacol. Rev. 67 (2), 280–309.
Syed, R.H., Gilliam, B.E., Moore, T.L., 2008. Rheumatoid factors and anticyclic citrullinated peptide antibodies in pediatric rheumatology. Curr. Rheumatol. Rep. 10 (2), 156.
Tang, M.J., Zhou, Z.B., 2013. Association of the CTLA-4+ 49A/G polymorphism with rheumatoid arthritis in Chinese Han population. Mol. Biol. Rep. 40 (3), 2627–2631.
Tsukahara, S., Iwamoto, T., Ikari, K., Inoue, E., Tomatsu, T., Hara, M., Momohara, S., 2008. CTLA-4 CT60 polymorphism is not an independent genetic risk marker of rheumatoid arthritis in a Japanese population. Ann. Rheum. Dis. 67 (3), 428–429.
Ueda, H., Howson, J.M., Esposito, L., Heward, J., Chamberlain, G., Rainbow, D.B., Gough, S.C., 2003. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature 423 (6939), 506–511.
Vaidya, B., Pearce, S.H., Charlton, S., Marshall, N., Rowan, A.D., Griffiths, I.D., Kendall-402 Taylor, P., Cawston, T.E., Young-Min, S., 2002. An association between the CTLA4 403 exon 1 polymorphism and early rheumatoid arthritis with autoimmune 404 endocrinopathies. Rheumatol. (Oxford) 41 (2), 180–183.
Van Belzen, M.J., Mulder, C.J., Zhernakova, A., Pearson, P.L., Houwen, R.H., Wijmenga, C., 2004. CTLA4+ 49 A/G and CT60 polymorphisms in Dutch coeliac disease patients. Eur. J. Hum. Genet. 12 (9), 782–785.
Wang, S.L., Chang, C.H., Hu, L.Y., Tsai, S.J., Yang, A.C., You, Z.H., 2014. Risk of developing depressive disorders following rheumatoid arthritis: a nationwide population-based study. PLoS One 9, e107791.
Yanagawa, T., Gomi, K., Nakao, E.I., Inada, S., 2000. CTLA-4 gene polymorphismin Japanese patients with rheumatoid arthritis. J. Rheumatol. 27 (12), 2740–2742.
Yokosuka, T., Kobayashi, W., Takamatsu, M., Sakata-Sogawa, K., Zeng, H., Hashimoto-Tane, A., Saito, T., 2010. Spatiotemporal basis of CTLA-4 costimulatory molecule-mediated negative regulation of T cell activation. Immunity. 33 (3), 326–339.
Zhou, C., Gao, S., Yuan, X., Shu, Z., Li, S., Sun, X., Xiao, J., Liu, H., 2021. Association between CTLA-4 gene polymorphism and risk of rheumatoid arthritis: a meta-analysis. Aging (Albany NY) 13 (15), 19397.