Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2021.82945.2271 Volume 30, Issue 2, March 2024
Manuscript ID ZUMJ-2106-2271
DOI 10.21608/ZUMJ.2021.82945.2271

ORIGINAL ARTICLE

Association of Solute Carrier 22 Member 4 Gene Polymorphism with Rheumatoid Arthritis

***Lamiaa AbdelWahab Mohammad*[1], *Nahla M. Gaballah*[2], *Aya A. ElShahawy*[1]*, *Saffaa M.Elalawi*[1]**

[1] *Clinical Pathology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt.*

[2] *Rheumatology and Rehabilitation Department, Faculty of Medicine, Zagazig University, Zagazig,Egypt.*

***Corresponding Author:** Aya AbdelMoneam ElShahawy, Assistant Lecturer of Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt.

E-mail: Aya_alshahawy@yahoo.com

ABSTRACT

Background: Rheumatoid arthritis (RA) is an autoimmune inflammatory joint disease that causes persistent inflammation, joint deterioration, severe damage, and restricted mobility. Its definite cause is unknown, but genetic and environmental factors are contributory. The study aimed to determine the association of *SLC22A4* polymorphism with the severity of rheumatoid arthritis in Zagazig University Hospitals.

Methods: Thirty-four RA cases diagnosed according to the criteria of the American College of Rheumatology (ACR) and 34 normal controls were enrolled in this study. All cases have given consent and detailed history. Clinical examination, plain x ray and laboratory investigations including erythrocyte sedimentation rate, C-reactive protein, anti-cyclic-citrullinated peptide antibodies and rheumatoid factor were performed. Disease activity score-28 (DAS-28) was assessed. The SLC22A4 *slc2F1* (rs2073838) and *slc2F2* (rs3792876) polymorphisms were genotyped by direct sequencing.

Results: The distribution of A alleles of *slc2F1* genotype in RA patients were two times than in control while distribution of T alleles of *slc2F2* genotype in RA patients were three times than in control but the difference was statistically non-significant ($p > 0.05$). No significant association between radiographic damage and *slc2F1/slc2F2* genotypes and alleles.

Conclusions: SLC22A4 variants, particularly *slc2F1/slc2F2*, does not affect RA susceptibility or severity in the studied RA patients as there were no significant differences in genotypic or allelic frequencies between RA patients and controls.

Key words: Rheumatoid arthritis, Solute carrier family 22 member 4, ergothioneine.

INTRODUCTION

Rheumatoid arthritis is a chronic autoimmune joint disorder with unknown etiology that affects about 1% of the global population. It is characterized by joint destruction and limited mobility [1].

The development of RA has been linked to both hereditary and environmental factors. The heritability of RA is around 60%, implying that genetic factors may play a slightly larger role in RA risk than environmental factors [2]. The main genetic factor for RA is the human leukocyte antigen (HLA)-DRB1 gene but the HLA genes account only for one-third of the genetic liability to the disease and non-HLA genes are also involved. Several non-HLA genes as fibrosis [4]. *SLC22A4* located at chromosome 5q31 which is linked to inflammatory bowel and allergic illnesses as it includes many T helper 2 type cytokines genes involved in immune and inflammatory systems [5]. OCTN1 is a co-susceptibility factors have been proposed including peptidyl arginine deiminase type 4 *(PADI4*), signal transducer and activator of transcription (*STAT4*), solute carrier 22-member 4 gene (*SLC22A4)* and runt-related transcription factor 1 genes [3].

SLC22A4 is one of the non-HLA genes linked to RA encodes organic cation transporter novel 1 (OCTN1) that transport ergothioneine which is a thiourea derivative of histidine and natural antioxidant acquired from food and enriched in some tissues by OCTN1 [3]. The antioxidant ergothioneine is responsible of protection from inflammation, oxidative stress, and more severe liver

transporter taking up both sodium ions and ergothioneine into cells. *SLC22A4* is present in lymphoid tissue and overexpressed in collagen-induced arthritis [6].

An intronic single-nucleotide polymorphism

(SNP) rs2073838 (denoted *slc2F1*) in a Japanese population was the first to link *SLC22A4* to RA susceptibility. Another intronic SNP, rs3792876 (*slc2F2*), was discovered to alter *RUNX1* binding and was closely linked with *slc2F1* [7]. *SLC22A4* expression is increased under inflammatory circumstances, but it is negatively controlled by *RUNX1*, and severe suppression of *SLC22A4* expression increases the risk of RA [8]. SNPs in *SLC22A4* have also been linked to Crohn's disease and psoriasis, both of which have a pathophysiology linked to inflammation and autoimmune, like RA [9]. The aim of the study was to determine the association of *SLC22A4* polymorphism with the severity of rheumatoid arthritis in Zagazig University Hospitals.

METHODS

Study design: This case-control study was carried out from 2016 to 2017 at Clinical Pathology, Rheumatology &Rehabilitation departments, Faculty of Medicine, Zagazig University Hospitals. The study included 68 subjects divided into 2 groups**.**

Cases group included 34 RA cases (28 females, 6 males). They were diagnosed according to the 2010 American College of Rheumatology (ACR) [10]. Patients with other autoimmune diseases were excluded.

Control group included 34 apparently healthy, age and sex-matched subjects serving as controls. They were 26 females, 8 males.

All cases were subjected to full history taking, clinical examination, radiological damage was assessed using modified Larsen scores to assess severity of rheumatoid arthritis [11] and RA disease activity was assessed by DAS-28 ESR [12]. DAS before and after 6 months from therapy with (prednisone, hydroquine and methotrexate), the difference between them are calculated to assess response to therapy according to the European League Against Rheumatism response criteria [13].

Routine laboratory tests including erythrocyte consideration. This research was carried out in agreement with the Statement of Helsinki.

Statistical analysis:

All information was gathered, tabulated, and analyzed using SPSS version 19. Independent samples Student's t-test was used to compare between two groups of normally distributed variables while Mann Whitney U test was used for non- normally distributed variables. Kruskall Wallis test was used to compare between more than two independent groups of non-normally distributed variables. Percent of categorical variables were compared using Chi-square test or Fisher's exact test when appropriate. Spearman's rank correlation coefficient was calculated

The significance level was set at $P < 0.05$.

sedimentation rate (ESR) first hour (N=2-7 mm) (Westergreen method), C- reactive protein (CRP) (N=1-5 mg/l) and rheumatoid factor (RF) (N=0-15 U/ml) determined by Roche Cobas Integra 400), Anti cyclic citrullinated peptide (anti-CCP) (N=0-17 U/ml) assessed by Roche Cobas e411.

Specific laboratory testing: Genotyping of (*slc2F1/slc2F2*) polymorphism in *SLC22A4* gene by direct sequencing. Genomic DNA was extracted from anticoagulated whole blood samples using (QIAamp DNA Blood Mini Kits). PCR amplicons were generated using the following primer pairs: for *slc2F1* (rs2073838) ,forward 5'- AGCAGATGGATGCCTGAGACC-3' and reverse 5'-TCTCTAGGTTTGGCAGGAAGAAC-3'; for *slc2F2* (rs3792876), forward 5'-GAACCCTAGTGGAGCTGCTG-3' and reverse 5'- CACTGAGAGCAGCAAGCAAG-3' using (ready Top Taq Master Mix Kit QIAGEN) [14]. PCR products were purified with QIAquick PCR Purification (QIAGEN) [15]. The concentration of purified PCR product was measured by the (Qubit ® 3.0 Fluorometer Invitrogen life technologies Thermo Fisher Scientific, Waltham, MA, USA) and DNA cycle sequencing with the forward primer using a BigDye Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA, USA) [16] then secondary purification by Big dye X terminator by (Big dye X terminator purification kit, Applied Biosystems). DNA sequencing was analyzed with Genetic Analyzer 3500 (Hitachi, Applied Biosystems, USA). Analysis of sequencing results by submitting sequences on National Center for Biotechnology Information (NCBI), nucleotide BLAST (Basic Local Alignment Search Tool) for detection of *SLC22A4* gene polymorphism.

Ethical Approvals: The study was approved by the "Institutional Review Board" (IRB) committee at Faculty of Medicine, Zagazig University. A written consent was taken from all subjects for ethical

RESULTS

The mean age of RA patients was 52.1±9.1 years and that of controls was 54.2 ± 7.2 years. Six males and 28 females' patients while control group included 8 males and 26 females with no statistically significant difference between cases and controls regarding age and sex. **Table (1)** showed that the patient group had a significantly higher level of ESR, CRP , rheumatoid factor and anti CCP antibodies (p =0.0001 for all). The most

frequent RA manifestation was morning stiffness (100%) and the least frequent was interstitial lung disease (2.9%). Basal DAS ranged between 3.1–6.6 with a mean of 5.5±0.7. As regard response to treatment, higher percentage of rheumatoid patients show moderate response to treatment (61.7%), while 23.6% had a good response and 14.7% had no response to therapy **Table (2)**. Levels of RF,CRP and Anti CCP levels were found to be significantly increased with increased x-ray grade with a p value < 0.05 for all **Table (3)**.

All genotypes for both SNPs examined were in Hardy-Weinberg equilibrium in all groups. Regarding the frequency of genotypes of *slc2F1*, 94% of rheumatoid arthritis patients and 97% of control group had GG genotype, the difference was statistically not significant (p >0.05). Considering *slc2F2* gene, 91% of rheumatoid arthritis patients and 97% of control group had CC genotype with no statistically significant difference (p >0.05). However, RA patients were two times more likely to have (GA) genotype of *slc2F1* (OR = 2.06) than control and three times more likely to have (CT) genotype of *slc2F2* (OR = 3.19) **Table (4).** There were no statistically significant association between *slc2F1* genotypes and anti-CCP, RF and ESR levels (p > 0.05). However, patients with the genotype (GG) had a mean CRP level of 13 mg/l, while those with the genotype (GA) had mean CRP level of 40 mg/l, the difference was statistically significant (p =0.03). Regarding *slc2F2* genotype, there was no statistically significant difference as regard laboratory findings in both *slc2F2* genotypes **Table (5).** Moreover, neither *sl2F1* nor *slc2F2* had significant relation with radiological grade of rheumatoid arthritis patients **Table (6).**

There was a positive significant correlation between X-ray grade and laboratory parameters including: CRP, RF and Anti CCP. Positive significant correlation was also found between basal DAS and RF. Negative significant correlation was found between response to treatment and laboratory parameters: CRP and anti CCP levels **Table (7) Figure(1)**.

Table 1 Demographic and laboratory findings of the studied groups

Variables	Patient group (No=34)	Controlgroup (No=34)	P- value
Age (years)±SD Median (range)	52.1±9.1 50(36-65)	54.2±7.2 55(40-65)	0.3**
Sex females /male's ratio	28/6(4.7)	26/8 (3.25)	0.5*
CRP (mg/L)			
Mean ± SD Median (range)	14.7±12 8.5(3-50)	2.7±1.3 2.5(1-5)	0.0001**
Anti-CCP (U/mL) Mean ± SD Median (range)	138.8±173 75(3-850)	5.6±2.3 6(2-11)	0.0001**
RF (U/mL) Mean ± SD Median (range)	96.1±87.6 55(7-300)	6.7±3 7(2-12)	0.0001**
ESR (mm/hr.) Mean ± SD Median (range)	36±16.5 30(15-75)	4±1.4 4(2-6)	0.0001**

***Chi square test ** Mann-Whitney test**

Table 2 Clinical parameters of rheumatoid arthritis patients

Clinical picture	rheumatoid arthritis (No=34)
Morning stiffness No (%)	34(100)
Subcutaneous nodulesNo (%)	10(29.4)
Eye drynessNo (%)	6(17.6)
Deformity No (%)	11(32.4)
Interstitial lung diseaseNo (%)	1(2.9)
Disease duration per yearsMean ± SD Median (range)	12±4.6 12(4-20)
Age of onset/year'sMean ± SD Median (range)	40±7.7 40 (26-59)

Clinical picture	rheumatoid arthritis (No=34)
Basal DAS\ Mean ± SD Median (range)	 5.5±0.7 5.5 (3.1-6.6)
Response to treatmentGood Moderate No response	8(23.6%) 21(61.7%) 5(14.7%)

Table 3 Relation of serological parameters and different radiological grades of rheumatoid arthritis

X-ray grade	RF u/ml of RA patients	Anti CCP u/ml of RA patients	CRP mg/L ofRA patients
Grade I Mean± SD	 11.5±0.7	 8.5±2.1	 7±2.8
Min-max	11-12	7-100	5-9
Grade II Mean ± SD Min-max	 27.5±23 7-60	 31±30 4-66	 7±4.2 3-15
Grade III Mean ± SD Min-max	 40.50±21 7-70	 51.5±25 3-70	 12.2±9 6-30
Grade IV Mean ± SD Min-max	 124.3±84.5 28-240	 249.5±269 50-850	 21.75±15 5-50
Grade V Mean ± SD Min-max	 153.4±91.2 31-300	 184±134 45-500	 18.8±11.9 5-45
***P	0.005	0.001	0.04

*** Kruskal Wallis Test

Table 4 Frequency distribution of *slc2F1* and *slc2F2* genotypes a mong rheumatoid arthritis patients and control group

SLC22A4	*Patient group No=34 No (%)*	*Control group No=34 No (%)*	*P	RA to control group		**P
				Odds ratio (OR)	C.I (95%)	
slc2F1 genotypes						
(GG)	32(94)	33(97)		2.06	(0.178 , 23.882)	0.281
(GA)	2(6)	1(3)	0.99			
Allele frequencies						
(G) ®	66(97)	67(98.5)		2.03		
(A)	2(3)	1(2.5)	0.99		(0.180 , 22.933)	0.283
slc2F2 genotypes						
(CC)	31(91)	33(97)		3.19		
(CT)	3(9)	1(3)	0.6		(0.315 , 32.356)	0.163
Allele frequencies						
(C) ®	65(95.6)	67(98.5)	0.6	3.09		
(T)	3(4.4)	1(1.5)			(0.314 , 30.498)	0.166

*Fisher Exact test OR; odds ratio, C.I: confidence interval, ® Reference

Table 5 Relation of slc2F1/slc2F2 genotype and laboratory findings of rheumatoid arthritispatients

Items	slc2F1 genotypes		**p	slc2F2 genotypes		**p
	GG=32 No(%)	GA=2No(%)		CC=1No(%)	CT=3 No(%)	
CRP						
Mean ±SD Median(range)	13±10.3 8(3-45)	40±14 40(30-50)	0.03	15±12.5 8(3-50)	10.3±5 11(5-15)	0.81
Anti CCP						
Mean ±SDMedian (range)	139±177 75(3-850)	130±98.9 130(60-200)	0.74	143±180 70(3-850)	91±22 100(66-108)	0.715
RF						
Mean ±SDMedian (range)	94.56±88.4 55(7-300)	120±98.9 120(50-190)	0.53	96.1±90.8 50(7-300)	95±53 70(60-156)	0.54
ESR						
Mean ± SD Median (range)	34±15 35(15-75)	60±21.2 60(45-75)	0.07	34±15 30(15-75)	55±23 60(30-75)	0.098

**** Mann-Whitney u test**

Table 6 Comparison of radiological grade of rheumatoid arthritis patients with slc2F1& slc2F2genotypes

X-raygrade	slc2F1 genotypesof rheumatoid arthritis patients		χ^2	p	slc2F2 gene of rheumatoid arthritispatients		χ^2	p
	GG=32 No(%)	GA=2 No(%)			CC=31 No(%)	CT=3 No(%)		
Grade I	2(6)	0	3.1	0.99	2(6)	0	1.4	0.99
Grade II	6(19)	0		0.99	5(16)	1(33)		0.90
Grade III	5(16)	1(50)		0.65	6(19)	0		0.99
Grade IV	7(22)	1(50)		0.84	7(23)	1(33)		0.99
Grade V	12(37)	0		0.82	11(36)	1(33)		0.99

Table 7 Correlation between X ray grade, Basal DAS and response to treatment with the laboratory findings

Variables	x ray grade		Basal DAS		Response to treatment	
	r	p	r	p	r	p
CRP	0.368	0.032	0.105	0.569	-0.455	0.009
RF	0.653	0.000	0.365	0.040	-0.214	0.240
Anti CCP	-0.693	0.000	-0.170	0.353	-0.401	0.023

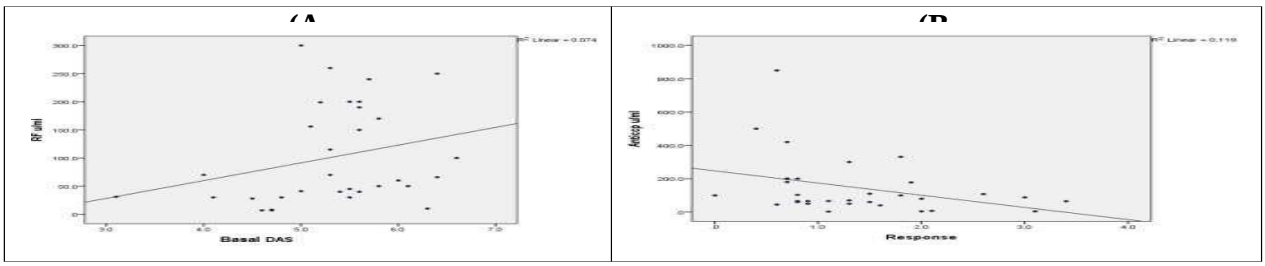

Fig 1 (A) Positive correlation between RF u/ml and basal DAS in RA patients.
(B) Negative correlation between response to treatment and Anti CCP in RA patients.

DISCUSSION

Rheumatoid arthritis is an autoimmune/inflammatory joint disease of unknown cause affecting about 1% of the population leading to joint destruction and disability. Its development is influenced by both hereditary and environmental factors [17]. The two autoantibodies mainly used for diagnosing/classifying RA are rheumatoid factor and anti-citrullinated protein antibodies. They precede the onset of disease symptoms and predict an aggressive disease course [18]. New treatment to stop RA before permanent joint destruction has been aided by the development of tools to evaluate disease activity and identify the presence or absence of remission [19]. The human organic cation/ergothioneine transporter 1 (*SLC22A4* gene) is responsible for the cellular uptake of substances, such as antioxidant found that the mean basal DAS was 4.0 and 27.5% were moderate response, while 49% had a good response. This slight difference may be attributed to the fact that the basal DAS in our study was higher indicating more activity of the disease.

The present study revealed that there was a positive significant correlation between X-ray grade and laboratory parameters including: CRP, RF and Anti CCP. Similarly, Bukhari et al. [23] reported that erosions and deformity were higher in anti-CCP positive patients than in anti-CCP negative patients. Han et al. [24] found that the RA erosion was significantly associated with rheumatoid factor positivity which was higher in erosive RA than non-erosive RA but not with anti- cyclic citrullinated peptide autoantibody positivity. These results were consistent with Mohammedet al. [25] who noticed that high titers of RF corresponded to severe erosive disease mandating aggressive therapy.

In similar observation Heidari [26] stated that CRP was significantly correlated with the severity of disease as well as radiographic changes**.** This came in agreement with Bay-Jensen et al.[27] who demonstrated that elevated CRP levels both at baseline and using time-integrated measures were correlated with rapid radiological progression and joint damage within 1 year.

The study showed that there was no statistically significant difference between RA patients and controls regarding the distribution of *slc2F1 and slc2F*2 genotypes or alleles in rheumatoid arthritis patients and control group. This agreed with Komlósi et al. [20] who found no statistically significant difference between RA patients and controls regarding the distribution of *slc2F2* genotypes or alleles.

ergothioneine. Intronic SNPs in *SLC22A4* inhibit *SLC22A4* transcription due to the stronger binding of the susceptibility allele to RUNX1 [20]. Regarding RA manifestations, the frequency of morning stiffness, deformity, subcutaneousnodules, eye dryness and interstitial lung disease were 100%, 32.4%, and 29.4%, 17.6% and 2.9% respectively.

A study by El Sherbiny [21] revealed that the most common extra-articular manifestations are subcutaneous rheumatoid nodules 45%, eye dryness 23% and interstitial lung disease 15%. This difference may be due to the number of studied cases.

In this study the mean basal DAS was 5.5 and 61.7% of rheumatoid patients showed moderate response to treatment and 23.6% had a good response. A study by Svensson et al. [22]

On the other hand of this study, Tokuhiro et al. [28] and Ren et al. [2] reported that there were variations in genotype distribution and allelic frequencies of slc2F1/slc2F2 polymorphisms between RA patients and controls. The presence of the slc2F1 A allele and the slc2F2 T allele increases the risk of RA by 2.03 and 1.93 times, respectively. This discrepancy in the results may beattributed to the difference in ethnicity, sample size, diet, and lifestyle factors. Moreover, the role of these genes in susceptibility to RA may be emphasized by the presence of environmental factors to which the Japanese and Chinese populations, but not other populations are exposed.

The present study revealed that the distribution of A alleles of *slc2F1* genotype in RA patients were two times than in control while distribution of T alleles of *slc2F2* genotype in RA patients were three times than in control. However, their odds ratios were more than 1 but there were not significant. This can be explained by the small sample size.

The present study found that neither *slc2F1* nor *slc2F2* genotypes had significant differences with the extra-articular manifestations, basal DAS, age of onset and response to treatment. This agreed with Newman et al. [29] who proved that there was no association between RA and the *slc2F1* risk allele regarding age of onset or severity markers including rheumatoid factor, nodules, and erosions.

Concerning the relation of the laboratory findings of rheumatoid arthritis patients with*slc2F1* and *slc2F2* genotypes, there was no significant difference except for the mean CRP level which was higher in GA genotypes rather than GG genotypes.

When comparing the radiological grade of rheumatoid arthritis patients and their *slc2F1* or *slc2F2* genotypes, there was no significant relation between them. These findings are in line with the study of Barton et al. [30] who denied presence of significant association between RA and *SLC22A4* SNPs in United Kingdom populations regarding RF, erosions, and carriage of shared- epitope alleles.

Barton et al. [30] explained the differences could be due to a Type II error (false-negative), a small sample size, or the possibility that linkage

In summary, this study revealed no significant association between RA susceptibility and *SLC22A4* gene variants in the Egyptian population. The *SLC22A4* gene may play a role in disease susceptibility only when ethnic-specific environmental or genetic variables are present, as some studies have shown and others have not.

CONCLUSIONS

There were no significant differences between RA patients and controls regarding genotypic or allelic frequencies indicating that *SLC22A4* polymorphisms, particularly *slc2F1/slc2F2*, have no effect on RA susceptibility or severity in the studied RA patients. So we recommend further studies with a larger sample size.

REFERENCES

1- **Amin DS, Ibrahim IK, Afifi AH, El-Hadidi AS, Al Sayed EH.** High resolution ultrasonography and power doppler in evaluation of disease activity of rheumatoid arthritis patients in clinical remission or low disease activity. Studies.2020; 7, 9.

2- **Ren, T. l., Han, Z. j., Yang, C. j., Hang, Y. x., Fang, D. y., Wang, K., et al.** Association of SLC22A4 gene polymorphism with Rheumatoid arthritis in the Chinese population. Journal of biochemical and molecular toxicology. 2014; 28(5):206-210.

3- **Chatzikyriakidou A, Voulgari PV, Lambropoulos A, Drosos AA.** Genetics in rheumatoid arthritis beyond HLA genes: what meta-analyses have shown? Seminars in arthritis and rheumatism. 2013: Elsevier. WB Saunders

4- **Samodelov SL, Kullak-Ublick GA, Gai Z, Visentin M.** Organic cation transporters in humanphysiology, pharmacology, and toxicology. International Journal of Molecular Sciences. 2020; 21(21):7890. 5- **Urcelay E, Concha E and Martínez A.** OCTN genes: Susceptibility genes for autoimmune diseases? Inmunología.2007; 26:87-96.

6- **Jerine Peter S, Panchal NK, Bapna I, Kulkarni S, Pratyusha S, Thiagarajan P, et al.** Protein modelling of the genes of Arthritis: A review. Int. Res. J. Pharm. 2020;11:13-18.

disequilibrium patterns differ in the Japanese and United Kingdom populations, and that the associated SNPs reported in the Japanese study are markers for another gene in the region, its role in RA susceptibility depend on the presence of environmental factors. Variations in races, diet and lifestyle factors present between them. These findings were matched with study of Han et al. [24] who showed that SNPs in *SLC22A4* introns were not associated with RA susceptibility or joint erosion.

7- **Plant D, Barton A, Thomson W, Ke X, Eyre S, Hinks A, Bowes J.** A re-evaluation of three putative functional single nucleotide polymorphisms in rheumatoid arthritis. Ann Rheum Dis. 2009;68:1373–1375.

8- **Yamada R, Tokuhiro S, Chang X, Yamamoto K.** SLC22A4 and RUNX1: identification of RA susceptible genes. Journal of molecular medicine. 2004;82(9):558-564.

9- **Jung ES, Park HJ, Kong K, Choi JH, Cheon JH.** Association study between OCTN1 functional haplotypes and Crohn's disease in a Korean population. The Korean Journal of Physiology & Pharmacology. 2017;21(1):11-17.

10- **Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham III CO, et al.** Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis & rheumatism. 2010;62(9):2569-2581.

11- **Larsen A.** How to apply Larsen score in evaluating radiographs of rheumatoid arthritis in long-term studies. J Rheumatol. 1995;22:1974-1975.

12- **Prevoo M, Van'T Hof MA, Kuper H, Van Leeuwen M, Van De Putte L, Van Riel P.** Modified disease activity scores that include twenty-eight-joint counts development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 1995;38(1):44-48.

13- **Salaffi F and Ciapetti A**: Clinical disease activity assessments in rheumatoid arthritis. International Journal of Clinical Rheumatology. 2013;8:347.

14- **Newman B, Wintle RF, van Oene M, Yazdanpanah M, Owen J, Johnson B, et al.** SLC22A4 polymorphisms implicated in rheumatoid arthritis and Crohn's disease are not associated with rheumatoid arthritis in a Canadian Caucasian population. Arthritis & Rheumatism: Official Journal of the American College of

Rheumatology. 2005;52(2):425-429.
15- **Carson S, Miller HB, Srougi MC, Witherow DS.** Molecular biology techniques: a classroom laboratory manual: Academic Press; 2019.
16- **Clark DP, Pazdernik NJ.** Chapter 4 - DNA Synthesis In Vivo and In Vitro. In: Clark DP, Pazdernik NJ, editors. Biotechnology (Second Edition). Boston: Academic Cell; 2016. p. 97-130.
17- **McInnes IB, Schett G.** The Pathogenesis of Rheumatoid Arthritis. New England Journal of Medicine. 2011;365(23):2205-2219.
18- **de Brito Rocha S, Baldo DC, Andrade LEC.** Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. Advances in rheumatology (London, England). 2019;59(1):2.
19- **Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein GS, et al.** Rheumatoid arthritis. Nature reviews Disease primers. 2018;4:18001.
20- **Komlósi K, Talián GC, Faragó B, Magyari L, Cserép V, Kovács B, et al.** No influence of SLC22A4 C6607T and RUNX1 G24658C genotypic variants on the circulating carnitine ester profile in patients with rheumatoid arthritis. Clinical and experimental rheumatology. 2008;26(1):61-66.
21- **ElSherbiny DA.** Frequency and Predictors of Extra-articular Manifestations in Patients with Rheumatoid Arthritis. The Egyptian Journal of Hospital Medicine. 2019;76(5):4062-4067.
22- **Svensson B, Schaufelberger C, Teleman A, Theander J, group ftBs.** Remission and response to early treatment of RA assessed by the Disease Activity Score. Rheumatology. 2000;39(9):1031-1036.
23- **Bukhari M, Thomson W, Naseem H, Bunn D, Silman A, Symmons D, et al.** The performance of anti–cyclic citrullinated peptide antibodies in predicting the severity of radiologic damage in inflammatory polyarthritis: results from the Norfolk Arthritis Register. Arthritis & Rheumatism: Official Journal of the American College of Rheumatology. 2007;56(9):2929-2935.
24- **Han T-U, Lee H-S, Kang C, Bae S-C.** Association of joint erosion with SLC22A4 gene polymorphisms inconsistently associated with rheumatoid arthritis susceptibility. Autoimmunity. 2015;48(5):313-317.
25- **Mohammed RHA, Farahat F, Kewan HH, Bukhari MA.** Predictors of European League Against Rheumatism (EULAR) good response, DAS-28 remission and sustained responses to TNF-inhibitors in rheumatoid arthritis: a prospective study in refractory disease. SpringerPlus. 2015;4(1):207.
26- **Heidari B.** Rheumatoid Arthritis: Early diagnosis and treatment outcomes. Caspian J Intern Med. 2011;2(1):161-170.
27- **Bay-Jensen AC, Platt A, Jenkins MA, Weinblatt ME, Byrjalsen I, Musa K, et al.** Tissue metabolite of type I collagen, C1M, and CRP predicts structural progression of rheumatoid arthritis. BMC Rheumatology. 2019;3(1):3.
28- **Tokuhiro S, Yamada R, Chang X, Suzuki A, Kochi Y, Sawada T, et al.** An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. Nature genetics. 2003;35(4):341-348.
29- **Newman B, Wintle RF, van Oene M, Yazdanpanah M, Owen J, Johnson B, et al.** SLC22A4 polymorphisms implicated in rheumatoid arthritis and Crohn's disease are not associated with rheumatoid arthritis in a Canadian Caucasian population. Arthritis & Rheumatology. 2005;52(2):425-429.
30- **Barton A, Eyre S, Bowes J, Ho P, John S, Worthington J.** Investigation of the SLC22A4 gene (associated with rheumatoid arthritis in a Japanese population) in a United Kingdom population of rheumatoid arthritis patients. Arthritis & Rheumatism. 2005;52(3):752-758.

To cite:

El Shahawy, A., Abdel Wahab Mohammad, L., Nahla M. Gaballah, N. N. M. G., M Elalawi, S. Association of Solute Carrier 22 Member 4 Gene Polymorphism with Rheumatoid Arthritis. *Zagazig University Medical Journal*, 2024; (533-540): -. doi: 10.21608/zumj.2021.82945.2271