

The association of TRAP1 gene and TNFSF4 gene polymorphisms with susceptibility of rheumatoid arthritis in a sample of Egyptian patients

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Received: 5 January 2023

Revised: 8 February 2023

Accepted: 15 February 2023

Published: 23 May 2023

Journal of The Arab Society for Medical Research 2023, 18:76–81

Background/aim

The disclosure of new gene polymorphisms and their association with rheumatoid arthritis (RA) susceptibility open new windows for better clarification of disease pathogenesis, leading to discovering new therapeutic targets. The present study aimed to explore the association of tumor necrosis factor receptor-associated protein 1 (TRAP1) gene rs8055172 and tumor necrosis factor superfamily number 4 (TNFSF4) gene rs1234315 with susceptibility of RA in a sample of Egyptian patients.

Patients and methods

This study included 200 RA patients from the Rheumatology Department Outpatients' Clinic of Kasr El Ainy Teaching Hospital and Centre of Medical Excellence of National Research Centre, Cairo, Egypt. The study also included 200 healthy participants with no family history of autoimmunity serving as a control group. Genotyping of the studied polymorphisms was done using real-time PCR technique.

Results

The control group showed no significant deviations from Hardy–Weinberg equilibrium regarding rs8055172 and rs1234315 ($P=0.6$ and 0.2 , respectively). Regarding genotypes of rs8055172, the CC homozygous genotype was more observed among patients. Therefore, the frequency of C allele is higher among RA patients compared with healthy controls ($P=0.001$). Logistic regression analysis of rs8055172 genotypes with susceptibility of RA was only significant under the recessive model, where patients carrying CC allele have higher susceptibility to develop RA ($P=0.001$, odds ratio=3.1) compared with patients carrying TT and CT allele. On the other hand, distribution of TNFSF4 (rs1234315) genotypes showed no significant difference between controls and RA group ($P=0.7$).

Conclusions

Our results indicate that the TRAP1 gene rs8055172 associates with RA in a population of Egyptians from Cairo, while TNFSF4 gene rs1234315 plays no role in disease susceptibility. A large-scale study to assess the association between TRAP1 gene polymorphism, TRAP1 mRNA expression, and TRAP1 protein level, is needed to clarify the role of TRAP1 gene polymorphism in RA pathogenesis.

Keywords:

polymorphisms and rheumatoid arthritis, rs1234315, rs8055172, TNFSF4 gene, TRAP1 gene

J Arab Soc Med Res 18:76–81

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1687-4293

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder [1]. It is a generalized chronic inflammatory reaction affecting ~1% of the adult population worldwide, leading to progressive destruction of the synovial joints associated with pain and deformity with subsequent reduced quality of life [2].

It is well known that genetic, epigenetic, and environmental factors can play significant roles in the pathogenesis and progression of this disease [1]. Recently, different studies have spotted the light on genetic factors in RA as it was documented that the

genetic factors alone can contribute in up to 60% of RA pathogenesis [3]. The discovery of new gene polymorphisms and their association with RA susceptibility open new windows for better clarification of disease pathogenesis, leading to discovering new therapeutic targets to improve disease outcome [3].

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Tumor necrosis factor receptor-associated protein-1 (TRAP1) gene encodes TRAP1 protein that belongs to the heat-shock protein-90 family [4]. It is a mitochondrial protein that has the ability to regulate mitochondrial function and stress responses and can protect against oxidative stress, which is increased in autoimmune diseases [5]. Different genetic studies suggested TRAP1 gene as candidate gene for a variety of autoimmune diseases, especially systemic lupus erythematosus (SLE) [6]. However, its association with RA is still underevaluated and no data are available in Egyptian population.

Tumor necrosis factor superfamily number-4 (TNFSF4) gene is a member of TNF receptor superfamily (TNFRSF) that encodes transmembrane protein that acts as a ligand for OX40 receptor (OX40 ligand, OX40L) [7]. TNFSF4 is mainly expressed on different activated immune cells, such as dendritic cells, macrophages, and T and B cells, playing a significant role in cytokine production, effector T-cell survival, memory cell formation, and differentiation of B cells. Therefore, many reports that showed the involvement of TNFSF4 in tissue inflammation may be via regulating the production of pro-inflammatory cytokines and mediators, suggesting its role in the occurrence of autoimmunity in both animal models and human diseases [8]. Many studies have discussed the association between TNFSF4 polymorphisms and other autoimmune diseases such as SLE, RA, and systemic sclerosis [9]. However, the results reported in these studies were not consistent. So far, there is no clear report on the association of TNFSF4 and RA in Egyptian population.

The present study aims to investigate the association of TRAP1 gene rs8055172 and TNFSF4 gene rs1234315 with susceptibility to RA in a sample of unrelated Egyptian patients from Cairo, Egypt.

Patients and methods

Patients and study design

A case-control study included 200 unrelated RA patients diagnosed according to the American College of Rheumatology 1987 revised criteria [10] recruited from the Rheumatology Department Outpatients' Clinic of Kasr El Ainy Teaching Hospital and Centre of Medical Excellence of National Research Centre. Patients with associated comorbidities, including other autoimmune diseases, diabetes, or malignancies, were excluded. The study also included 200 healthy age-matched and sex-matched volunteers with no family history of autoimmunity serving as a control group.

Complete history-taking and thorough clinical examination with musculoskeletal examination and assessment of tender and swollen joints in addition to routine labs were done and disease activity was assessed using disease activity score 28 (DAS28) [11] and clinical disease activity index (CDAI) [12].

Ethical consideration

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki in 2015. This study has been approved by the local Ethics Committee of National Research Centre (NRC), Cairo, Egypt with approval number 19047, a written informed consent was provided by each participant prior to their inclusion in the study.

Sample collection

Venous blood samples were collected from all participants in the following tubes: (a) EDTA vacutainer tube used for complete blood picture, erythrocyte sedimentation rate (ESR), and genotyping analysis and (b) plain vacutainer that was left to clot and serum was separated for routine laboratory workup.

Routine laboratory tests

Complete blood picture was done using Sysmex xs-500i Hematology Analyzer (Sysmex, Kobe, Japan), ESR was measured by the Westergren method. Serum concentrations of rheumatoid factor (RF) and C-reactive protein were assayed by Mispa i2 semi-automated nephelometer using Aggape kits (AGAPPE Diagnostic, Switzerland GmbH), alanine transaminase, aspartate transaminase, and creatinine were measured using XL 300-ERBA Mannheim autoanalyzer (ERBA Diagnostics, Mannheim, Germany), and anticyclic citrullinated peptides (ACCP) were measured by Cobas 6000 analyzer series (Roche Diagnostics, Mannheim, Germany).

Genotyping analysis for TRAP1 and TNFSF4 gene polymorphisms

Whole-blood samples were used for genomic DNA extraction by QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) following user's manual. The extracted DNA samples were stored at -20°C until analyzed. *TRAP1* rs8055172 and *TNFSF4* rs1234315C/T polymorphisms were genotyped by the TaqMan allelic discrimination method on LightCycler 480 Real-Time PCR System (Roche Diagnostics, Basel, Switzerland) following the manufacturer's protocol. All primers and probes were designed by the Applied Biosystems (Foster City,

California, USA) (TaqMan SNP Genotyping Assay, Cat no: 4351379). PCR condition was as follows: an initial activation step at 95°C for 10 min, followed by 40 cycles of denaturation for 15 s at 95°C, and annealing/extension for 1 min at 60°C. Fluorescence data collection is performed at the extension step and the final products were analyzed by LightCycler 480 Real-Time PCR System programmed software [2]. To ensure quality control, negative and positive controls were cogenotyped with each assay in addition to randomly selected 10% of samples measured in duplicates with concordance rate 100%.

Statistical analysis

Data analysis was done using SPSS (IBM Corp, Armonk, NY, USA), version 22 software. Qualitative data were described as frequencies and quantitative data were described as mean±SD. The χ^2 test was used to compare qualitative parameters and Student's *t* test was used to compare quantitative data. The Hardy–Weinberg equilibrium of the studied SNPs was detected among healthy controls using the χ^2 test. The logistic regression analysis was used to evaluate associations between the studied SNPs and RA with calculation of the odds ratios and 95% confidence intervals and after adjustment of traditional risk factors. In addition, the power of the study was calculated for rs11614913 association with T1DM, using the Power Calculations Program (PS Version 3.1.2).

Results

The present results indicated that there were no statistical differences between healthy controls and the studied patients regarding age (44.4±12.9, 45.5±11.4, *P*=0.300) and sex (188 females/12 males and 180 females/20 males, respectively, *P*=0.200). Other characteristics of the studied RA patients such as family history, disease duration, and disease activity, including DAS28 and CDAI, in addition to laboratory results of hemoglobin, ESR, total leukocytic count, platelets, aspartate transaminase, alanine transaminase, creatinine, RF, C-reactive protein, and ACCP, are all shown in Table 1.

The control group showed insignificant deviations from Hardy–Weinberg equilibrium regarding rs8055172 and rs1234315 (*P*=0.600 and 0.200, respectively). Regarding genotypes of TRAP1 gene (rs8055172), the TT homozygous and CT heterozygous genotypes were observed more frequently in the control group, while CC homozygous genotype was more observed among

patients. Therefore, the frequency of C allele is higher among RA patients compared with healthy controls (Table 2). Logistic regression analysis of rs8055172 genotypes with susceptibility of RA was only significant under recessive model, where participants carrying CC allele have higher susceptibility to develop RA compared with participants carrying TT and CT allele (Table 2). On the other hand, distribution of TNFSF4 (rs1234315) genotypes showed no significant difference between controls and RA group (Table 2).

Regarding TRAP1 gene (rs8055172), different patients' parameters, including hemoglobin levels, platelet count, serum creatinine, RF, and ACCP, showed no statistical differences between patients with CC genotype and other patients, while levels of ESR, total leukocytic count, DAS, and CDAI showed statistically significant differences between the two groups (Table 3). Power analysis of the study yielded a statistical power of 98% for association of TRAP1 gene (rs8055172) with RA.

Discussion

RA is one of the most common chronic inflammatory arthropathies all over the world. It is characterized by progressive joint damage and associated different systemic disorders, establishing an urgent medical problem for healthcare systems worldwide [1]. It is a multifactorial disease triggered mainly by genetic

Table 1 Main characteristics of the studied rheumatoid arthritis patients

Variables	RA patients (N=200)
Sex (female/male) ^a	180/20
Age (years) ^b	45.5±11.4
Family history of RA ^a (positive/negative)	29/171
Disease duration (years) ^b	6.9±5.7
Hemoglobin (g/dl) ^b	12±1.2
ESR (mm) ^b	38±24
TLC (×10 ³ /μl) ^b	8±6.8
Platelets (×10 ³ /μl) ^b	291±79
AST (IU/l) ^b	20.3±8.3
ALT (IU/l) ^b	22.5±7.6
Creatinine (mg/dl) ^b	0.7±0.2
RF positivity ^a (positive/negative)	122/78
CRP positivity ^a (positive/negative)	113/87
ACCP positivity ^a (positive/negative)	72/128
DAS ^b	4.5±1.7
CDAI ^b	18.1±15.2

^aData presented as frequencies. ^bData presented as mean±SD. ACCP, anticyclic citrullinated peptide; ALT, alanine transaminase; AST, aspartate transaminase; CDAI, composite index for quantifying disease activity; CRP, C-reactive protein; DAS, disease activity score; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; TLC, total leukocytic count.

Table 2 Comparisons and different models of genotypes and allele frequencies between the control and rheumatoid arthritis groups

SNP ID	Variables	Controls (N=200)	RA (N=200)	P value	OR (CI)
TRAP1 gene (rs8055172)	Genotypes				
	TT	120	110	0.001	1.6 (1.1–2.2)
	CC	18	48		
	CT	62	42		
	Alleles (2n)				
	T allele	302	262	0.001	
	C allele	98	138		
	Additive model: TT			Reference	
	CC			0.001	2.9 (1.5–5.3)
	CT			0.200	0.7 (0.4–1.1)
	Dominant model: (CC+CT/TT)			0.300	1.2 (0.8–1.8)
	Recessive model: (CC/CT+TT)			0.001	3.1 (1.7–5.7)
TNFSF4 gene (rs1234315)	Genotypes				
	CC	62	67		
	TT	24	20	0.700	1 (0.8–1.4)
	CT	114	113		
	Alleles (2n)				
	C	162	153	0.500	
	T				
	Additive model: CC			Reference	
	TT			0.400	0.7 (0.3–1.5)
	CT			0.600	0.9 (0.5–1.4)
	Dominant model: (TT+CT/CC)			0.500	0.8 (0.5–1.3)
	Recessive model: (TT/CT+CC)			0.400	0.8 (0.6–1.5)

CI, confidence interval; OR, odds ratio.

Table 3 Comparisons of different parameters in patients with TRAP1 (rs8055172) CC genotype and patients with other genotypes

Parameters	CC genotype (N=48)	TT+CT genotypes (N=152)	P value
ESR (mm) ^b	48.2±29.3	33.5±22.3	0.002
Hemoglobin (g/dl) ^b	12.3±1.2	12±1.2	0.100
TLC (×10 ³ /μl) ^b	6.6±2	8.5±7.7	0.006
Platelets (×10 ³ /μl) ^b	286±76	293±81	0.600
Serum creatinine (mg/dl) ^b	0.7±0.19	0.75±0.2	0.800
RF positivity ^a (positive/negative)	24/24	98/54	0.070
ACCP positivity ^a (positive/negative)	22/26	50/102	0.100
DAS ^b	5.1±1.8	4.2±1.6	0.001
CDAI ^b	22.8±17	16.7±14	0.020

^aData presented as frequencies. ^bData presented as mean±SD. ACCP, anticyclic citrullinated peptide; CDAI, composite index for quantifying disease activity; DAS, disease activity score; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; TLC, total leukocytic count.

factors. Identifying of new genes predisposes and affects disease course and elucidates understanding of RA molecular pathogenesis and signaling pathways [2]. To our knowledge, this is the first study demonstrating the association of TRAP1 gene

rs8055172 and TNFSF4 gene rs1234315 with the RA in Egyptian population.

TRAP1 gene is situated on chromosome 16p13 and encodes for TRAP1 protein that is a highly conserved chaperone molecule belonging to the heat-shock protein-90 family [6]. The major and the most important function of TRAP1 protein is protecting cells from damage after stress by playing a significant role in refolding-impaired protein and signal transduction [4]. Based on this important protein function and previous reports that suggested the 16p13 loci as important loci carrying genes playing a role in autoimmune diseases, recent studies have spotted the light on the role of TRAP1 gene polymorphisms in autoimmune diseases [13]. Fismen *et al.* [14] have demonstrated that altered expression of TRAP1 mRNA is associated with the progression of lupus nephritis in both human and murine models. Moreover, Li *et al.* [6] have suggested the association of TRAP1 gene polymorphism with susceptibility of SLE. Our study is the first study that suggested the association between TRAP1 gene rs8055172 and susceptibility of RA, in agreement with the established role of TRAP1 in protection against oxidative stress, the main hallmark in pathogenesis of RA [4,13]. Therefore, we suggest

that this SNP may have an impact on the expression of TRAP1 mRNA or affects TRAP1 protein level and function. Our data also suggested the C allele as risk allele for RA, logistic regression analysis was significant in the recessive model where individuals carrying CC allele have higher risk of susceptibility than others carrying TT or even CT genotype. In other words, the T allele may be a protective allele against RA and individuals carrying T allele (CT or TT) had lower susceptibility to develop RA. Moreover, we demonstrated the association of C allele of TRAP1 gene rs8055172 with markers of disease activity such as DAS and CDAI in partial agreement with Li *et al.* [6] who reported the association of TRAP1 gene polymorphisms with the response to glucocorticoids in patients with SLE. It is also worth to be noted that altered expression of TRAP1 mRNA was considered to be related to cancer progression and multidrug resistance in different human malignancies, supporting our results about the association of TRAP1 gene polymorphism with markers of disease activity [15,16].

TNFSF4 encodes for the protein OX40L that is expressed on different immune cells, provides a costimulatory molecule for different immune signals such as T-cell proliferation, survival, and cytokine production [17]. Different reports have focused on the role of soluble OX40L and OX40L expression in T cells of synovial fluid among patients with RA [18,19], suggesting the critical role of OX40L in the development of RA by stimulating Th1-type autoimmune response [19]. Moreover, the association between TNFSF4 SNPs and SLE and Sjögren's syndrome has been established by many studies and reports [9]. However, very few reports have studied the association of TNFSF4 DNA polymorphisms with RA. In the current study, we found no significant differences in genotypic and allelic frequencies of TNFSF4 rs1234315 between the two studied groups. These findings are contradicting with Ramírez-Bello *et al.* [17] that reported the association of rs1234315 polymorphisms with RA in 459 Mexican patients. Xu *et al.* [9] reported the association of TNFSF4 rs1234315 SNPs with susceptibility of RA in 210 Chinese patients. This contradiction between our results and other results might be ascribed to differences in ethnic origins, sample size, and associated epigenetic and environmental factors.

Conclusions

Our results indicate that the TRAP1 gene rs8055172 associates with susceptibility of RA in a population

from Cairo of Egypt, while TNFSF4 gene rs1234315 plays no role in disease susceptibility. An extensive study to assess the association between TRAP1 gene polymorphism, TRAP1 mRNA expression, and TRAP1 protein level, is needed to clarify for better clarification of the role of TRAP1 gene polymorphism in RA pathogenesis.

Acknowledgments

The authors are deeply appreciating for all members of the Rheumatology Department Outpatients' Clinic of Kasr El Ainy Teaching Hospital and Centre of Medical Excellence of National Research Centre, at Cairo, Egypt for their help to complete this work.

Authors' contribution: all authors contributed to the study conception and design, material preparation, and data collection. All authors revised and approved the final paper.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

- 1 El-Lebedy D, Hussein J, Ashmawy I, Mohammed AM. Serum level of neopterin is not a marker of disease activity in treated rheumatoid arthritis patients. *Clin Rheumatol* 2017; 36:1975–1979.
- 2 El-Lebedy D, Raslan H, Ibrahim A, Ashmawy I, Abd El-Aziz S, Mohammed A. Association of stat4 RS7574865 and PTPN22 RS2476601 polymorphisms with rheumatoid arthritis and non-systemically reacting antibodies in Egyptian patients. *Clin Rheumatol* 2017; 36:1981–1987.
- 3 Giannini D, Antonucci M, Petrelli F, Billa S, Alunno A, Puxeddu I. One year in review 2020: pathogenesis of rheumatoid arthritis. *Clin Exp Rheumatol* 2020; 38:387–397.
- 4 Ramos Rego I, Santos Cruz B, Ambrósio AF, Alves CH. TRAP1 in oxidative stress and neurodegeneration. *Antioxidants* 2021; 10:1829.
- 5 Liu L, Zhang L, Zhao J, Guo X, Luo Y, Hu W, *et al.* Tumor necrosis factor receptor-associated protein 1 protects against mitochondrial injury by preventing high glucose-induced MPTP opening in diabetes. *Oxid Med Cell Longev* 2020; 2020:1–17.
- 6 Li S, Sun X, Xu J, Gu Y, Liu S, Xu S, *et al.* Association study of TRAP1 gene polymorphisms with susceptibility and glucocorticoids efficacy of systemic lupus erythematosus. *Gene* 2018; 671:117–126.
- 7 Wang J-M., Yuan Z-C., Huang A-F., Xu W-D. Association of TNFSF4 RS1234315, rs2205960 polymorphisms and systemic lupus erythematosus susceptibility: a meta-analysis. *Lupus* 2019; 28:1197–1204.
- 8 Fu Y, Lin Q, Z-rong Z. Association of TNFSF4 polymorphisms with systemic lupus erythematosus: a meta-analysis. *Advances in Rheumatology* 2021; 61:59.
- 9 Xu J, He Y, Wang J, Li X, Huang L, Li S, *et al.* Influence of the TNFSF4 rs1234315 polymorphism in the susceptibility to systemic lupus erythematosus and rheumatoid arthritis. *Hum Immunol* 2019; 80: 270–275.
- 10 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthr Rheumat* 1988; 31:315–324.
- 11 Prevoo ML, Van'T Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, Van Riel PL. Modified disease activity scores that include twenty-eight-joint counts development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthr Rheumat* 1995; 38:44–48.

- 12 Aletaha D, Nell VPK, Stamm T, Uffmann M, Pflugbeil S, Machold K, *et al.* Acute phase reactants add little to composite disease activity indices for rheumatoid arthritis: validation of a clinical activity score. *Arthr Res Therapy* 2005; 7:R796–R806.
- 13 Zhang Z, Cheng Y, Zhou X, Li Y, Gao J, Han J, *et al.* Polymorphisms at 16p13 are associated with systemic lupus erythematosus in the Chinese population. *J Med Genet* 2010; 48:69–72.
- 14 Fismen S, Thiyagarajan D, Seredkina N, Nielsen H, Jacobsen S, Elung-Jensen T, *et al.* Impact of the tumor necrosis factor receptor-associated protein 1 (TRAP1) on renal DNase1 shutdown and on progression of murine and human lupus nephritis. *Am J Pathol* 2013; 182:688–700.
- 15 De Andrade W, Braga L, Gonçalves N, Maria Silva L, Lopes Da Silva Filho A. HSPA1A, HSPA1L and TRAP1 heat shock genes may be associated with prognosis in ovarian epithelial cancer. *Oncol Lett* 2020; 19:359–367.
- 16 Purushottam Dharaskar S, Paithankar K, Kanugovi Vijayavit A, Shabbir Kara H, Amere Subbarao S. Mitochondrial chaperone, TRAP1 modulates mitochondrial dynamics and promotes tumor metastasis. *Mitochondrion* 2020; 54:92–101.
- 17 Ramírez-Bello J, Jiménez-Morales S, Barbosa-Cobos RE, Sánchez-Zauco N, Hernández-Molina G, Luria-Pérez R, *et al.* TNFSF4 is a risk factor for rheumatoid arthritis but not for primary Sjögren's syndrome in the Mexican population. *Immunobiology* 2022; 227:152244.
- 18 Laustsen JK, Rasmussen TK, Stengaard-Pedersen K, Petersen KH, Hetland ML, Østergaard M, *et al.* Soluble OX40L is associated with presence of autoantibodies in early rheumatoid arthritis. *Arthr Res Ther* 2014; 16:474.
- 19 Yoshioka T, Nakajima A, Akiba H, Ishiwata T, Asano G, Yoshino S, *et al.* Contribution of OX40/OX40 ligand interaction to the pathogenesis of rheumatoid arthritis. *Eur J Immunol* 2000; 30:2815–2823.