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Association between TLR-2 and TLR-9 gene polymorphisms (rs5743708 and rs5743836) and susceptibility to psoriatic arthritis in Egyptian patients

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Abstract

Psoriasis (PsO) is a chronic immune-mediated disease of the skin. Psoriatic arthritis (PsA) is a prevalent chronic inflammatory disease that is associated with joint destruction and disability. The presence of PsO is the single greatest risk factor for the development of PsA. Toll-like receptors (TLRs) are trans-membrane proteins coded by the toll genes family. They are expressed in different cell types including immune and non-immune cells. Polymorphisms in TLR genes that lead to changes in these receptors or interfere with the transcription rates of their messenger ribonucleic acid (mRNA), may be involved in the chronic inflammatory immune response observed in PsA. This study involved 50 patients with PsA, 50 patients with cutaneous PsO and 50 age and sex matched normal subjects as controls. We aimed to assess TLR-2 (rs5743708) and TLR-9 (rs5743836) gene polymorphisms as potential risk factors for PsA in Egyptian patients with cutaneous PsO. Genotyping and allele frequencies were performed using Real Time polymerase chain reaction (qRT-PCR). Toll-like receptor-2 (TLR-2) rs5743708 and TLR-9 rs5743836 polymorphisms were associated with increased risk of PsO as an autoimmune disease, however they were not related to increase susceptibility to PsA in this cohort study of Egyptian patients.

Keywords: Psoriasis, Psoriatic Arthritis, Toll-like receptor-2 (TLR-2) gene polymorphism (rs5743708) and TLR-9 (rs5743836), gene polymorphism.

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Introduction

Psoriasis (PsO) is a chronic immune-mediated disease with a strong genetic predisposition and wide dermatological manifestations.[1] It is characterized by well-circumscribed, red papules or plaques with a grey or silvery-white, dry scale. It affects the scalp, elbows, knees, lumbosacral area, and the body folds. It is mediated by effector T cell activation and deregulated inflammatory cytokine expression.[2]

Psoriatic arthritis (PsA), or broader term psoriatic disease, refers to an inflammatory disorder that affects numerous organs, including the skin and joints. The prevalence of

PsA in Egypt was found to be 30%, with a mean age of 45.48 ±10.79 years. It is associated with several comorbidities and can significantly worsen health-related quality of life.[3] PA has several patterns of joint involvement, including axial and peripheral disease, and other extra-articular manifestations like enthesitis, dactylitis, and uveitis.[4]

The presence of PsO is the single greatest risk factor for the development of PsA; the presence of PsA among first-degree relatives and psoriatic nail dystrophy also increases the risk of PsA. In 2021, Urruticoechea-Arana and co-workers reported that up to 30% of patients with PsO will develop PsA during the disease course, usually within 10 years of their skin disease's first manifestations.[5]

Genetics, environmental factors, and immune-mediated inflammation are involved in the pathogenesis of PsA. However, their precise mechanisms are complex and incompletely understood. There are no specific markers or autoantibodies for the diagnosis of PsA: it is seronegative for both rheumatoid factor (RF) and antibodies to cyclic citrullinated peptides.

Different variables have been identified to predict whether a person with PsO may develop PsA, including clinical characteristics of PsO such as the severity of PsO or the presence of certain features of PsO (e.g., scalp lesions, nail disease, and intergluteal/perianal PsO), the presence of soluble biomarkers, like highly sensitive C-reactive protein (CRP) or certain susceptibility genes, like toll-like receptors (TLRs) gene polymorphism.[7] However, their prediction role is unclear. On the other hand, different questionnaires and strategies can be used to detect PsA in patients with PsO.[5]

TLRs are important initiators of the immune response, both innate and acquired. Evidence suggests that gene polymorphisms within TLRs cause malfunctions of certain key TLR-related signaling pathways which subsequently increase the risk of autoimmune diseases. [8] The mechanisms by which TLR polymorphisms modify the functions of TLRs are not fully understood. They may affect receptor function through their effects on TLR expression, localization, trafficking and signaling.[9]

The TLR-2 rs5743708 is a missense variant which affects the structure of the TLR-2 protein in the intracellular region and generates a nonfunctional protein, due to replacement of the amino acid arginine for glycine at position 753 of the protein (Arg753Gln). This change in TLR-2 reduces the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway and compromises the intracellular signaling cascade. [10]

The TLR-9 rs5743836 is a polymorphism in the TLR-9 gene promoter region and has been linked to autoimmunity. This polymorphism is a regulatory region variant of the TLR-9 gene. The C allele of rs5743836 polymorphism is located in the regions of CTF-binding sites and sites of several transcription factors (TF). The rs5743836∗C creates new NF-κB sites in the promoter region of TLR-9 gene, and it has been observed that this allele generates new multiple binding sites for different TFs.[11]

The TLR-2 rs5743708 and TLR-9 rs5743836 polymorphisms were associated with the risk of spondylarthritis (SpA) development, including PsA, these mutations may lead to significant changes in the innate and adaptive immune response profile, as well as in the maintenance of the regulation of immunological mechanisms.[12] Considering these, the study by Thibodaux et al., 2018, suggested that these polymorphisms contribute to potentiate the T helper (Th)1, Th2, and Th17 immune response seen in SpA, which may confer to individuals carrying the polymorphisms a predisposition to the development of SpA.[13]

There is a lack of studies associating genetic polymorphisms in TLRs and PsA polymorphisms Therefore, we aimed to assess the association between these variants of TLR-2 and TLR-9 and occurrence of PsA in Egyptian patients with PsO, and to determine their relation to PsO (being an autoimmune disease) as a possible predisposing factor for autoimmunity

Subjects and Methods

This case-control study was conducted at Alzahraa University Hospital during the period from October 2021 to February 2022.The study enrolled 150 subjects of both sexes (56 males

and 94 females). Their ages ranged from 19 years to 60 years.

Participants were divided into 3 groups. The first included 50 patients with PsA, recruited from the outpatient clinic of the Department of Rheumatology and Rehabilitation. The second included 50 patients with cutaneous PsO, recruited from the outpatient clinic of the Department of Dermatology, and the third group included 50 normal individuals enrolled as controls from many colleagues who are well known to be normal.

Patients included in our study were PA patients satisfying the 2006 Classification Criteria for PsA (CASPAR)[14] and assessed by the same rheumatologist. Patients with chronic plague PsO were clinically diagnosed by the same dermatologist. Patients who had other autoimmune diseases, other infectious skin lesions and other types of seronegative arthropathy were excluded from our study.

Assessment and Procedures

A complete history was taken from all patients with special emphasis on age, onset, course and duration of both PsO and PsA, history of previous chronic diseases and family history of PsO. General examination assessed the general condition of the patient and local examination including skin and joint examination.

All patients in the study underwent the following laboratory investigations. For this, a venous blood sample (6 mL) was collected from each subject. Of these, 2 ml were placed into one EDTA (K3EDTA) vacutainer for complete blood count (CBC) and for Erythrocyte sedimentation rate (ESR). Another sample of 2 ml was placed into a second EDTA vacutainer and kept in deep freezer (at -80 C) for the polymerase chain reaction (PCR) test.

CBC was done immediately using fully automated cell counter (Sysmex KX21N, Kobe, Japan), according to the manufacturer's instructions. The ESR was done by using the Westergren method.

The remaining blood sample (2 ml) was placed in a sterile vacutainer with a clot activator and left to clot for 30 minutes. The samples were centrifuged at 742-1660 xg for 20 minutes. The separated serum was used for CRP measurement using commercial kits (Catalogue No.07876033190, COBAS INTEGRA, Kit supplied by Roche Diagnostics, USA), RF using Latex agglutination method (Catalogue No.518002Kit supplied by Egyptian Company For Biotechnology, Germany) and for Antinuclear antibody (ANA) measurement using the indirect immunofluorescence technique (IIF) using commercial kits (Catalogue No.708101, Kit supplied by Inova Diagnostics, Inc, USA), according to the manufacturer's instructions.

Molecular Analysis of Blood Sample

Genomic DNA extraction

Genomic DNA was extracted from blood samples using commercially available kits (Lot. No.00672154, Thermo-Fisher Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit, Baltics UAB V. A. Graiciuno 8, LT-02241 Vilnius, Lithuania), according to the manufacturer's instructions.

Assessment of genomic gDNA concentration and purity

DNA concentration was assessed spectrophotometrically using a spectrophotometer (QIAxpert, Qiagen, Hilden, Germany). The concentrations of DNA and proteins were determined by measuring the absorbance at 260 nm (A260) and at 280 nm (A280), respectively. The ratio of absorbance at 260 and 280 nm was used to assess DNA purity. DNA concentration was estimated by measuring the absorbance at 260 nm, adjusting the A260 measurement for turbidity (measured by absorbance at 320 nm), multiplying by the dilution factor, and using the relationship that an A260 of 1.0 = 50µg/ml pure dsDNA (DNA concentration (µg/ml) = (A260 reading –A320 reading) ×dilution factor×50µg/ml).

Genotyping of the TLR-2 rs5743708 and TLR-9 rs5743836 by quantitative Real Time polymerase chain reaction (qRT-PCR)

Two TaqMan® SNP genotyping assays for TLR-2 rs5743708 and TLR-9 rs574383 and genotyping master mix (Applied Biosystems, Thermo Fisher Scientific, USA) were used. Each genotyping

assay contained sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest, two TaqMan® minor groove binder (MGB) probes with non-fluorescent quenchers (NFQ): one VIC™-labelled probe to detect the allele 1 sequence and one FAM™-labelled probe to detect allele 2 sequence. The Context Sequence [VIC/FAM] for TLR-2 rs5743708 and TLR-9 rs574383 were (ATTCCCCAGCGCTTCTGCAAGCTGC [A/G]GAAGATAATGAACACCAAGACCTAC and TGGCCATTGTTATTTTTGTTTTACA[G/C]CTGAAGAAACTGAGACTCCATAAGT), respectively.

The genotyping PCR reaction was conducted in 100 µl PCR tubes. Each 20 µl total reaction volume contained 10 µl of TaqMan Genotyping Master Mix (Lot. No. 00728613 Applied Biosystems, Thermo Fisher Scientific, USA), 0.5 µl of TaqMan® SNP Genotyping Assay, and 9.5 µl of diluted DNA template (5.5 µl nuclease free water, (Lot No. 01069419), and 4 µl template). The PCR process consisted of 40 cycles, each of an initial denaturation at 95°C for 15 seconds to separate the nucleic acid double chain, then combined annealing of primers and extension of new strands at 60°C for 1 minute. During PCR amplification process, data were collected and read based on fluorescence signals using a Rotor Gene real-time system (QIAGEN, Hilden, Germany). The allelic discrimination data were plotted as a comparison of allele 1 (VICTM dye) and allele 2 (FAMTM dye) using the real-time PCR instrument software (QIAGEN, Hilden, Germany). Every specimen is represented as a separate curve that represents the amplified alleles and the specimen genotype (Figures 1 and 2).

Statistical Analysis

The statistical analysis was performed using the statistical package for social sciences (SPSS), version 23.0 (IBM Inc., Chicago, Illinois, USA). Normally distributed quantitative data are represented as the mean and standard deviation (SD). The student's t-test was used for comparison between two groups, whereas the Mann–Whitney test for non-parametric data. Qualitative variables are presented as frequencies and percentages. The Fisher's exact test or the chi-square (χ^2) test was employed to compare qualitative variables. TLR-2 and TLR-9 genetic variants were assessed for Hardy-Weinberg equilibrium (HWE) with a chi-square test before genotypic and allelic disease correlation analysis. The odds ratio (OR) of genotypes between groups was assessed using logistic regression. The level of statistical significance was considered at $p < 0.05$.

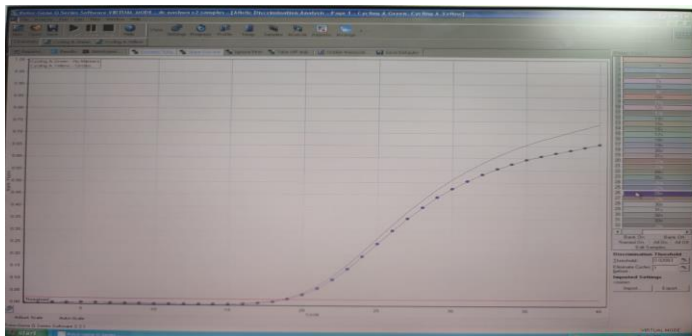

Figure 1. An amplification plot showing a case with heterozygous genotype for TLR-2 gene (rs5743708) and TLR-9 gene (rs5743836). It shows the amplification of both reference and alternative alleles.

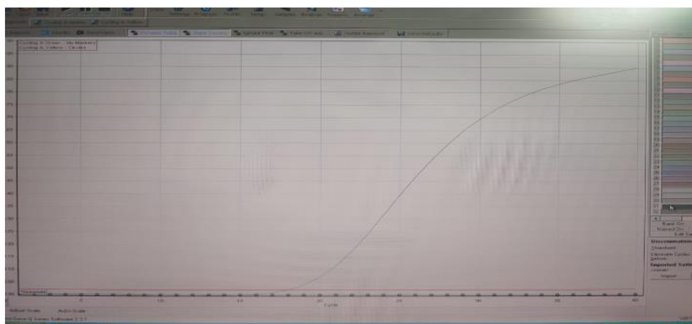

Figure 2. An amplification plot showing a case with homozygous genotype for TLR-2 gene (rs5743708) and TLR-9 gene (rs5743836). It shows the amplification of one allele only.

Results

The characteristics of the study patients and controls are demonstrated in Table 1. Table 2 displays comparison between clinical data for the PsA group and Cutaneous Pso group. It shows a statistically significant difference in associated medical diseases diabetes mellitus (DM) and hypertension (HTN) (p<0.05) between PsA group and Cutaneous PsO group in which PsA patients revealed more association. Also, there was a statistically significant increase in duration of PsO (p=0.020) and PASI score (*p*<0.001) when PsA patients are compared to PsO patients.

Table 1. Comparison of demographic data between the 100 patients and 50 controls.

Demographic data	Patients Group	Control Group	*p*-value
Age (years)			
Mean ±SD	43.14±11.82	45.24±10.59	NS^{t}
Range	19-60	19-59	
Sex			
Female	40 (40.0%)	23 (46.0%)	NS ^{x2}
Male	60 (60.0%)	27 (54.0%)	

p > 0.05 is not significant (NS). T: T test. x2: Chi-square test.

Table 2. Comparison between clinical data of the 50 psoriatic arthritis (PsA) group and the 50 Cutaneous psoriasis (PsO) group.

Clinical data	PsA Group	Cutaneous PsO Group	*p*-value
Associated medical diseases			
DM	8 (16.0%)	0 (0.0%)	0.003^{x2}
HTN	8 (16.0%)	2 (4.0%)	0.047^{x2}
Gout	2 (4.0%)	0 (0.0%)	NS^{x2}
Family History of PSO	9 (18.0%)	4 (8.0%)	NS^{x2}
Duration of PsO (years)			
Mean ±SD	11.88±9.01	8.40±5.14	0.020^{U}
Range	4-38	1-20	
PASI Score			
Mean ±SD	14.50±7.51	8.30±5.82	<0.001 ^{U}
Range	2-28	2-28	

U: Mann-Whitney test; x2: Chi-square test; *p*-value <0.05 S; DM (Diabetes Mellitus), HTN (Hypertension), PASI (PsO area and severity index). *p* > 0.05 is not significant (NS).

Table 3 displays comparison between laboratory data of the patients and the control groups. It shows a statistically significant increase regarding white blood cells (WBCs) when patients group compared to control group (*p*<0.001), while there was a statistically significant decrease in hemoglobin (Hb) and platelet (PLT) count when the patients group compared to the control group (*p*<0.001).

Table 3. Comparison between laboratory data in the 100 patients and 50 control groups.

Laboratory data	Patients Group	Control Group	*p*-value
White blood cells (WBCs)			
Mean±SD	8.35±2.29	6.60±0.98	<0.001
Range	4.8-14.2	4.8-8.7	

Table 3. Continued.

Laboratory data	Patients Group	Control Group	*p*-value
Hemoglobin (Hb)			
Mean±SD	12.15±1.53	13.63±1.23	<0.001
Range	9.7-15.8	12-16	
Platelet (PLT) count			
Mean±SD	247.24±64.40	290.90±57.72	<0.001
Range	120-450	218-450	

t: Independent Sample t-test, $p<0.05$ is significant.

Table 4 displays the comparison in genotypes and allelic distribution of TLR-2 SNP (rs5743708) between the patients and control groups. It shows 3 genotypes of TLR-2 (rs5743708) (AA, AG and GG). AG genotype was significantly higher in all patients (24%) than controls (4%) ($p = 0.006$) and associated with increased risk of development of PsO [OR= 8.23,95% CI, 1.86-36.46; p=0.006]. Also, A allele showed a statistically significant increase in the patients' group (18%) compared to control group (2%) [OR= 10.76, 95% CI, 2.53-45.66; p<0.001]. The genotype frequencies were significantly higher in the patients than controls under dominant model of inheritance [OR= 10.29, 95% CI, 2.53-45.66; p=0.002]. While the recessive model of inheritance showed insignificant difference between the two groups ($p = 0.189$).

Table 4. Comparison in the genotypes and allelic distribution of TLR-2 SNP (rs5743708) between patients and controls.

Gene polymorphisms rs5743708	Patients Group (n=100)	Control Group (n=50)	OR (C.I. 95%)	*p*-value
Genotyping				
GG	70 (70.0%)	48 (96.0%)	Reference	
AG	24 (24.0%)	2 (4.0%)	8.23 (1.86-36.46)	0.006
AA	6 (6.0%)	0 (0.0%)	---	NS
Alleles frequency				
G	164 (82.0%)	98 (98.0%)	Reference	<0.001
A	36 (18.0%)	2 (2.0%)	10.76 (2.53-45.66)	
Dominant model				
GG	70 (70.0%)	48 (96.0%)	Reference	0.002
AG+AA	30 (30.0%)	2 (4.0%)	10.29 (2.35-45.08)	
Recessive model				
GG+AG	94 (94.0%)	50 (100.0%)	Reference	NS
AA	6 (6.0%)	0 (0.0%)	---	

$p > 0.05$ is not significant (NS).

Table 5 displays comparison in the genotypes and allelic distribution of TLR-9 SNP (rs5743836) between patients and control groups. It shows 3 genotypes of TLR-9 (rs5743836) (TT, TC and CC). TC genotype was significantly higher in the patients than controls and associated with increased risk of development of PsO [OR= 3.53, 95% CI, 1.43-8.73; p=0.006]. Also, C allele showed a statistically significant increase in patients' group (49%) compared to control group (11%) [OR= 2.63, 95% CI, 1.2-5.31; p<0.007]. The genotype frequencies were significantly higher in patients than controls under dominant model of inheritance [OR= 13.29, 95% CI, 1.45-7.52; p=0.005]. While the recessive model of inheritance showed insignificant difference between the two groups ($p = 0.472$).

Table 5. Comparison in genotypes and allelic distribution of TLR-9 SNP (rs5743836) between patients and control groups.

Gene polymorphisms rs5743836	Patients Group (n=100)	Control Group (n=50)	OR (C.I. 95%)	*p* value
Genotyping				
TT	58 (58.0%)	41 (82.0%)	Reference	
TC	35 (35.0%)	7 (14.0%)	3.53 (1.43-8.73)	0.006
CC	7 (7.0%)	2 (4.0%)	2.47 (0.49-12.52)	NS
Alleles frequency				
T	151 (75.5%)	89 (89.0%)	Reference	0.007
C	49 (24.5%)	11 (11.0%)	2.63 (1.20-5.31)	
Dominant model				
TT	58 (58.0%)	41 (82.0%)	Reference	0.005
TC+CC	42 (42.0%)	9 (18.0%)	3.29 (1.45-7.52)	
Recessive model				
TT+TC	93 (93.0%)	48 (96.0%)	Reference	NS
CC	7 (7.0%)	2 (4.0%)	1.80 (0.36-9.03)	

OR: Odds Ratio & C.I.: Confidence Interval, $p > 0.05$ is not significant (NS).

Table 6 displays comparison of genotypes and allelic distribution of TLR-2 SNP (rs5743708) between the studied groups. Comparing the PsA group to the control group revealed a statistically significant increase in A allele in PsA group (9%) (p=0.047). While there was no statistically significant difference observed in genotypes and both models of inheritance between the two groups (p> 0.05). Comparing the Cutaneous PsO group to the control group revealed a statistically significant increase in AG genotypes, A allele and dominant model of inheritance in the Cutaneous PsO group (p<0.001). While there was no statistically significant difference in genotypes and recessive model of inheritance between the two groups (p > 0.05). Comparing PsA to Cutaneous PsO revealed a statistically significant increase in AG genotypes, A allele and dominant model of inheritance in the PsA group (p<0.05). While there was no statistically significant difference in the recessive model of inheritance between the two groups (p > 0.05).

Table 6. Comparison between genotypes and allelic distribution of TLR-2 SNP (rs5743708) in the studied groups.

Gene polymorphism rs5743708	PsA Group (n=50)	Cutaneous PsO Group (n=50)	Control Group (n=50)	PsA vs. Control	PsO vs. Control	PsA vs. PsO
				OR (C.I. 95%), *p*-value	OR (C.I. 95%), *p*-value	OR (C.I.95%), *p*-value
Genotyping						
GG	43 (86.0%)	27 (54.0%)	48 (96.0%)	Reference	Reference	Reference
AG	5 (10.0%)	19 (38.0%)	2 (4.0%)	2.79 (0.52-15.13), *p*(NS)	16.88 (3.65-78.1), *p*<0.001	0.16 (0.06-0.50); *p*=0.002
AA	2 (4.0%)	4 (8.0%)	0 (0.0%)	p(NS)	*p*=0.067	0.31 (0.05-1.83); *p*(NS)

Table 6. Continued.

Gene polymorphism rs5743708	PsA Group (n=50)	Cutaneous PsO Group (n=50)	Control Group (n=50)	PsA vs. Control	PsO vs. Control	PsA vs. PsO
				OR (C.I. 95%), *p*-value	OR (C.I. 95%), *p*-value	OR (C.I.95%), *p*-value
Alleles frequency						
G	91 (91%)	73 (73%)	98 (98%)	Reference	Reference	Reference
A	9 (9%)	27 (27%)	2 (2%)	4.85 (1.02-23.03); *p*=0.047	18.12 (4.18-78.66); *p*<0.001	0.27 (0.12-0.60); *p*=0.002
Dominant model						
GG	43 (86%)	27 (54%)	48 (96%)	Reference	Reference	Reference
AG+AA	7 (14%)	23 (46%)	2 (4%)	3.91 (0.77-19.83); *p*(NS)	20.44 (4.47-93.46); *p*<0.001	20.44 (4.47-93.47); *p*<0.001
Recessive model						
GG+AG	48 (96%)	46 (92%)	50 (100%)	Reference	Reference	Reference
AA	2 (4%)	4 (8%)	0 (0%)	*p*(NS)	*p*(NS)	0.48 (0.08-2.74); *p*(NS)

OR: Odds Ratio, C.I.: Confidence Interval; PsA (Psoriatic Arthritis), $p > 0.05$ is not significant (NS).

Table 7 displays comparison in genotypes and allelic distribution of TLR-9 SNP (rs5743836) between all studied groups. Comparing the PsA group to the control group revealed no statistically significant difference in genotypes, alleles and model of inheritance between the two groups ($p > 0.05$). Comparing the Cutaneous PsO group to the Control group revealed a significant increase in TC genotypes, C allele (31%) and dominant model of inheritance in the Cutaneous PsO group ($p < 0.001$). While there was no significant difference in recessive model of inheritance between the two groups ($p > 0.05$). Comparing the PsA group to the cutaneous PsO group revealed a statistically significant increase in the C allele (31%) and dominant model of inheritance in the PsO group ($p < 0.05$). While there was no-significant difference in TC genotype and in the recessive model of inheritance ($p > 0.05$).

Table 7. Comparison between genotypes and allelic distribution of TLR-9 SNP (rs5743836) in the studied groups.

Gene polymorphism rs5743836	PA Group (n=50)	Cutaneous PsO Group (n=50)	Control Group (n=50)	PsA vs. Control	Cutaneous PsO vs. Control	PsA vs. Cutaneous PsO
				OR (C.I. 95%), *p*-value	OR (C.I. 95%), *p*-value	OR (C.I. 95%), *p*-value
Genotyping						
TT	34 (68.0%)	24 (48.0%)	41 (82.0%)	Reference	Reference	Reference
TC	14 (28.0%)	21 (42.0%)	7 (14.0%)	2.41 (0.87-6.66); *p*(NS)	5.13 (1.99-13.83); *p*<0.001	0.47 (0.2-1.11); *p*(NS)
CC	2 (4.0%)	5 (10.0%)	2 (4.0%)	1.2 (0.16-9); *p*(NS)	4.27 (0.77-23.74); *p*(NS)	0.28 (0.05-1.58); *p*(NS)

Table 7. Continued.

Gene polymorphism rs5743836	PA Group (n=50)	Cutaneous PsO Group (n=50)	Control Group (n=50)	PsA vs. Control	Cutaneous PsO vs. Control	PsA vs. Cutaneous PsO
				OR (C.I. 95%), *p*-value	OR (C.I. 95%), *p*-value	OR (C.I. 95%), *p*-value
Alleles frequency						
T	82 (82.0%)	69 (69.0%)	89 (89.0%)	Reference	Reference	Reference
C	18 (18.0%)	31 (31.0%)	11 (11.0%)	1.78 (0.79-3.98); *p*(NS)	3.64 (1.71-7.74); *p*<0.001	0.49 (0.25-0.95); *p*=0.034
Dominant model						
TT	34 (68.0%)	24 (48.0%)	41 (82.0%)	Reference	Reference	Reference
TC+CC	16 (32.0%)	26 (52.0%)	9 (18.0%)	2.15 (0.84-5.46); *p*(NS)	4.94 (1.98-12.26); *p*<0.001	0.43 (0.19-0.98); *p*=0.044
Recessive model						
TT+TC	48 (96.0%)	45 (90.0%)	48 (96.0%)	Reference	Reference	Reference
CC	2 (4.0%)	5 (10.0%)	2 (4.0%)	1 (0.14-7.39); *p*(NS)	2.67 (0.49-14.44); *p*(NS)	0.38 (0.07-2.03); *p*(NS)

OR: Odds Ratio & C.I.: Confidence Interval, *p* > 0.05 is not significant (NS); PsA: Psoriatic Arthritis.

Table 8 displays the association between genotypes of TLR-2 SNP (rs5743708) and all studied parameters in the PsA group. It shows an association between genotypes of TLR-2 SNP (rs5743708) and age, PASI score, DAPSA score, dactylitis, morning stiffness of axial joint, and PLT (*p*<0.05). However, there was no significant relation between other studied parameters and genotype of TLR-2 (rs5743708) gene polymorphism (*p*>0.05).

Table 8. Association between genotypes of TLR-2 SNP (rs5743708) and all studied parameters in the PsA group.

PsA Group	Genotyping (rs5743708)			*p*-value
	GG (n=43)	AG (n=5)	AA (n=2)	
Age (years)	48.30±8.69	41.60±11.84	30.50±0.71	0.013^{F}
Sex				
F	22 (51.2%)	1 (20.0%)	1 (50.0%)	NS^{x2}
M	21 (48.8%)	4 (80.0%)	1 (50.0%)	
Associated medical diseases				
DM	8 (18.6%)	0 (0.0%)	0 (0.0%)	NS^{x2}
HTN	8 (18.6%)	0 (0.0%)	0 (0.0%)	NS^{x2}
Gout	2 (4.7%)	0 (0.0%)	0 (0.0%)	NS^{x2}
Family history of PSO	7 (16.3%)	1 (20.0%)	1 (50.0%)	NS^{x2}
Duration of PsO (years)	12.16±9.36	11.60±7.70	6.50±0.71	NS^{H}
PASI Score	13.22±7.04B	21.16±5.95 A	25.25±3.89 A	0.007^{H}
Enthesitis	25 (58.1%)	3 (60.0%)	2 (100.0%)	NS^{x2}
Dactylitis	9 (20.9%) B	2 (40.0%) B	2 (100.0%) A	0.034^{x2}

Table 8. Continued.

PsA Group	Genotyping (rs5743708)			*p*-value
	GG (n=43)	AG (n=5)	AA (n=2)	
Associated medical diseases				
Morning stiffness of axial joint	9 (20.9%) C	3 (60.0%) B	2 (100.0%) A	0.013^{x2}
Morning stiffness of peripheral joint	16 (37.2%)	3 (60.0%)	2 (100.0%)	NS^{x2}
Nail changes	27 (62.8%)	3 (60.0%)	2 (100.0%)	NS^{x2}
Duration of Arthritis (years)	3.91±2.07	4.20±1.30	5.50±0.71	NS^{x2}
DAPSA score	21.60±11.62 B	50.20±22.19 A	52.50±4.95 A	<0.001^{F}
ANA	5 (11.6%)	1 (20.0%)	1 (50.0%)	NS^{x2}
WBCS	6.66±1.00	6.16±0.91	6.50±0.99	NS^{F}
Hb	12.50±1.73	13.38±0.75	13.05±1.34	NS^{F}
PLT	281.81±54.13 C	339.20±49.28 B	365.50±64.35 A	0.016^{F}

Values in each row which have different letters are significantly different (*p*<0.05). F: One Way analysis of variance & Multiple comparison between groups through Post Hoc test: Tukey's test H: Kruskal Wallis test & Multiple comparison between groups through Mann-Whitney test x2: Chi-square test; *p* > 0.05 is not significant (NS).
DM (Diabetes Mellitus), HTN (Hypertension), PASI (PsO area and severity index), ANA (Antinuclear Antibodies), DAPSA (Disease Activity in Psoriatic Arthritis).

Table 9 displays the association between genotypes of TLR-2 SNP (rs5743708) and all studied parameters in the Cutaneous PsO group. It shows an association between genotypes of TLR-2 (rs5743708) and PASI score (*p*<0.001) and WBCs (*p*=0.043). However, there was no significant relation between the other parameters studied and genotype of TLR-2 SNP (rs5743708) (*p*>0.05).

Table 9. Association between genotypes of TLR-2 SNP (rs5743708) and all studied parameters in the Cutaneous PsO group.

Cutaneous PsO Group	Genotyping (rs5743708)			*p*-value
	GG (n=27)	AG (n=19)	AA (n=4)	
Age (years)	39.07±14.41	39.32±9.29	41.50±17.71	NS^{F}
Sex				
F	8 (29.6%)	7 (36.8%)	1 (25.0%)	NS^{x2}
M	19 (70.4%)	12 (63.2%)	3 (75.0%)	
Associated medical diseases				
DM	0 (0.0%)	0 (0.0%)	0 (0.0%)	NS^{x2}
HTN	0 (0.0%)	1 (5.3%)	1 (25.0%)	NS^{x2}
Gout	0 (0.0%)	0 (0.0%)	0 (0.0%)	NS^{x2}
Family history of PsO	4 (14.8%)	0 (0.0%)	0 (0.0%)	NS^{x2}
Duration of PsO (years)	7.44±4.03	9.95±6.54	7.50±3.00	NS^{H}
PASI Score	4.85±2.15C	11.05±5.47B	18.53±6.32A	<0.001^{H}
ANA	0 (0.0%)	0 (0.0%)	0 (0.0%)	NS^{x2}
WBCS	7.07±0.47A	6.82±0.33B	6.63±0.28C	0.043^{F}
Hb	12.42±1.65	12.88±1.64	12.58±2.04	NS^{F}
PLT	278.96±45.55	307.68±60.07	291.75±108.23	NS^{H}

Values in each row which have different letters are significantly different at (*p*<0.05) F: One Way analysis of variance & Multiple comparison between groups through Post Hoc test: Tukey's test H: Kruskal Wallis test & Multiple comparison between groups through Mann-Whitney test x2: Chi-square test; *p* > 0.05 is not significant (NS); DM: Diabetes mellitus, HTN: Hypertension, PASI: PsO area and severity index, ANA: Anti-nuclear antibodies.

Table 10 displays the association between genotypes of TLR-9 SNP (rs5743836) and all studied parameters in the PsA group. It shows an association between genotypes of TLR-9

(rs5743836) and dactylitis and morning stiffness of peripheral joint (p <0.05). However, there was no significant relation between other parameters studied and genotype of TLR-9 (rs5743836) (p >0.05).

Table 10. Association between genotypes of TLR-9 SNP (rs5743836) and all studied parameters in the PsA group.

PsA Group	Genotyping (rs5743836)			*p*- value
	TT (n=34)	TC (n=14)	CC (n=2)	
Age (years)	46.12±9.77	49.14±9.81	45.00±0.00	NS^{F}
Sex				
F	17 (50.0%)	5 (35.7%)	2 (100.0%)	NS^{x2}
M	17 (50.0%)	9 (64.3%)	0 (0.0%)	
Associated medical diseases				
DM	8 (23.5%)	0 (0.0%)	0 (0.0%)	NS^{x2}
HTN	8 (23.5%)	0 (0.0%)	0 (0.0%)	NS^{x2}
Gout	2 (5.9%)	0 (0.0%)	0 (0.0%)	NS^{x2}
FHx of PsO	8 (23.5%)	1 (7.1%)	0 (0.0%)	NS^{x2}
Duration of PsO (years)	12.29±10.05	11.43±6.82	8.00±0.00	NS^{H}
PASI Score	13.04±7.02	17.95±8.32	15.00±0.00	NS^{H}
Duration of Arthritis (years)	4.32±2.20	3.36±1.28	3.00±0.00	NS^{H}
Enthesitis	18 (52.9%)	10 (71.4%)	2 (100.0%)	NS^{x2}
Dactylitis	6 (17.6%) B	5 (35.7%) B	2 (100.0%) A	0.022^{x2}
Morning stiffness of axial joint	8 (23.5%)	4 (28.6%)	2 (100.0%)	NS^{x2}
Morning stiffness of peripheral joint	10 (29.4%) C	9 (64.3%) B	2 (100.0%) A	0.020^{x2}
Nail changes	19 (55.9%)	11 (78.6%)	2 (100.0%)	NS^{x2}
DAPSA score	23.74±15.20	30.50±19.11	25.50±0.71	NS^{H}
ANA	3 (8.8%)	4 (28.6%)	0 (0.0%)	NS^{x2}
WBCs	6.74±0.99	6.15±0.89	7.40±0.00	NS^{F}
Hb	12.42±1.75	13.22±1.36	11.50±0.00	NS^{F}
PLT	289.38±60.26	289.00±55.62	330.00±0.00	NS^{F}

Values in each row which have different letters are significantly different (p<0.05) F: One Way analysis of variance & H: Kruskal Wallis test; x2: Chi-square test; p > 0.05 is not significant (NS); DM: Diabetes mellitus, HTN: Hypertension, FHx: Family History, PASI: PsO area and severity index, ANA: Anti-nuclear Antibodies, DAPSA: Disease Activity in Psoriatic Arthritis.

Table 11 displays an association between the genotyping of TLR-9 SNP (rs5743836) and all studied parameters in the Cutaneous PsO group. It shows an association between genotypes of TLR-9 (rs5743836) and PASI score (p =0.002). However, there was no significant relation between the other studied parameters and genotype of TLR-9 (rs5743836) in the Cutaneous PsO group (p >0.05).

Table 11. Association between genotyping of TLR-9 gene polymorphism SNP (rs5743836) and all studied parameters in the Cutaneous PsO group.

Cutaneous PsO Group	Genotyping (rs5743836)			*p*- value
	TT (n=24)	TC (n=21)	CC (n=5)	
Age (years)	37.67±13.82	40.14±12.13	44.20±9.78	NS^{F}
Sex				
F	10 (41.7%)	5 (23.8%)	1 (20.0%)	NS^{x2}
M	14 (58.3%)	16 (76.2%)	4 (80.0%)	

Table 11. Continued.

Cutaneous PsO Group	Genotyping (rs5743836)			*p*- value
	TT (n=24)	TC (n=21)	CC (n=5)	
Associated medical diseases				
DM	0 (0.0%)	0 (0.0%)	0 (0.0%)	NS^{x2}
HTN	0 (0.0%)	2 (9.5%)	0 (0.0%)	NS^{x2}
Gout	0 (0.0%)	0 (0.0%)	0 (0.0%)	NS^{x2}
FHx of PsO	1 (4.2%)	3 (14.3%)	0 (0.0%)	NS^{x2}
Duration of PsO (years)	7.13±3.88	9.57±5.91	9.60±6.58	NS^{H}
PASI Score	5.45±3.17 B	10.66±7.10 A	12.06±3.11 A	0.002^{H}
ANA	24 (100.0%)	21 (100.0%)	5 (100.0%)	NS^{x2}
WBCS	6.93±0.40	6.92±0.48	7.08±0.41	NS^{F}
Hb.	12.81±1.70	12.39±1.70	12.56±1.34	NS^{F}
PLT	277.63±37.15	305.00±69.93	295.40±79.38	NS^{F}

Values in each row which have different letters are significantly different ($p<0.05$) F: One Way analysis of variance & Multiple comparison between groups through Post Hoc test: Tukey's test H: Kruskal Wallis test & Multiple comparison between groups through Mann-Whitney test x2: Chi-square test; $p > 0.05$ is not significant (NS). DM: Diabetes mellitus), HTN: Hypertension, FHx: Family History, PASI: PsO area and severity index, ANA: Anti-nuclear Antibodies.

Discussion

Our study aimed to assess TLR-2 (rs5743708) and TLR-9 (rs5743836) gene polymorphisms as potential risk factors for PsA in Egyptian patients with cutaneous PsO and to determine their relation to PsO (being an autoimmune disease) as a possible predisposing factor for autoimmunity.

In the present study, genotyping of TLR-2 gene (rs5743708) revealed three genotypes (GG, GA, AA). AG genotype was significantly higher in patients (24%) than controls (4%) and associated with increased risk of PsO [OR = 8.23, 95% CI, 1.86–36.46; *p*< 0.006]. Also, A allele showed a statistically significant increase in the patients' group (36%) compared to control group (2%) and associated with 10-fold increased risk of PsO [OR = 10.76, 95% CI, 1–45.66; *p* < 0.001]. The same results were obtained when we compared the PsO group to the control group where both AG genotype and A allele were significantly higher in the PsO group than the control group [OR= 16.88, 95%CI, 3.65-78.1; *p*<0.001].

Reinforcing this result, on comparing PsO group to PsA group, we revealed a statistically significant increase in (AG) genotype and in (A) allele (*p*<0.002) in the PsO group when compared to PsA group. This indicates that TLR-2 rs5743708∗A variant is a susceptibility factor to PsO but not to PsA and the analyzed polymorphism is linked to autoimmunity.

Several studies have suggested that genetic variants of TLRs play a crucial role in different autoimmune diseases,[8] such as type 1 diabetes mellitus,[15] Graves' disease,[16] rheumatoid arthritis,[17] systemic lupus erythematous,[18] and multiple sclerosis.[19]

The genetic variability of TLR-2 was also featured with an involvement in susceptibility in inflammatory diseases,[20] The study by Zhou et al., 2023, showed significant correlations between TLR-2 gene rs5743708 and Atopic Dermatitis .[21] In addition, the study by El-Nabi et al., 2020[22] reported that the Arg753Gln variant in the TLR-2 gene was linked to cytomegalovirus infection in Egyptian bone marrow recipients. They showed that 85% of the patients screened were G/G homozygous, 15% G/A heterozygous, however no patients with homozygous (A/A).

Moreover, the study by Alhababi et al., 2023,[23] reported that TLR-2 rs5743708 and TLR-9 rs5743836 variants might be independent risk factors influencing susceptibility and severity to Coronavirus disease 2019, but they do not seem to be associated with markers of thrombosis. However, the true impact of the TLR polymorphisms on PsO susceptibility and disease course was unclear.[24]

In a study done by Zablotna et al., 2017, the results did not show any association between

TLR-2 rs5743708∗A polymorphism and PsO susceptibility, however they demonstrated the potential influence of these polymorphisms on clinical presentation and the clinical course of the disease. Their study included 175 patients with PsO and 170 healthy controls. They found that genotype and allele frequencies for Arg753Gln TLR-2 and –1237 T/C TLR-9 gene polymorphisms in patients with PsO were similar to those in healthy controls. However, the patients with late onset of PsO were more likely to carry allele G in the Arg753Gln TLR-2 polymorphism, whereas allele T in the –1237 T/C TLR-9 polymorphism was more frequent in the patients with early onset of PsO.[25]

In our study, when comparing genotypes and allelic frequencies of TLR-2 (rs5743708) in the PsA group to the control group, we revealed a statistically significant increase in A allele in the PsA group (9%) in comparison to control group (2%) (*p*=0.047). This goes with findings of the study by Oliveira-Toré et al., 2019, who reported that the TLR-2 gene rs5743708∗A polymorphism increased the chance of developing PsA. Furthermore, the presence of homozygote (rs5743708∗A/A) was not observed among the controls.[12] We attributed the significant increase in (A) allele in PsA group to the presence of underlying PsO. Our concern about their results is that they compared PsA group to control group without considering the presence of underlying PsO (as an autoimmune disease) as a possible underlying risk factor. They did not compare PA patients to patients with PsO as we did. Their control group included healthy subjects, and this relation could be either due to the presence of PsO or due to arthritis as an inflammatory condition associated with PsO.

In our study, in PsA patients, we found an association between genotypes of TLR-2 SNP (rs5743708) and age, PASI score, DAPSA score dactylitis, morning stiffness of axial joint and PLT counts (*p*<0.05). This comes in agreement with that of Kaeley et al., 2018, who reported that dactylitis is associated with more erosive forms of PsA[26] and with finding by Yan et al., 2018, who revealed significant association between PsA and severity of PSO (PASI score) (*p* < 0.05).[27] Also, the study by Kaeley and Gurjit, 2020, reported that enthesitis is associated with more erosive forms of PsA[28]. In 2015, Noreen and his co-workers reported that the SNPs neither cause disease nor are absolute indicators of PsA disease development, but they can help to determine the likelihood that someone will develop a particular illness.[29]

Regarding TLR-9, genotyping of rs5743836 revealed three genotypes (TT, TC and CC). TC genotype was significantly higher in PsA patients (35%) than controls (14%) and associated with increased risk of PsO [OR= 3.53, 95% CI, 1.43-8.73; *p* = 0.006]. Also, C allele showed a statistically significant increase in the PsA patients' group (49%) compared to control group (11%) and associated with increased risk of PsO [OR= 2.63, 95%CI, 1.2-5.3; *p*< 0.007]. Similar results were obtained when we compared the PsO group to the control group, we revealed a statistically significant increase in TC genotype and C allele (31%) in the PsO group when compared to control group (11%) and associated with increased risk of PsO [OR=5.13, 95%CI, 1.99-13.83; *p*<0.001].

Reinforcing this result, comparing PsA group to PsO group, we revealed a statistically significant increase in C allele in the PsO group when compared to the PsA group (*p*=0.034). This indicates that TLR-9 rs5743836∗C variant is a susceptibility factor to PsO but not to PsA and the analyzed polymorphism is linked to autoimmunity.

Our result came in disagreement with those reported by Zablotna et al., 2017, where they observed no association between TLR-9gene rs5743836∗C polymorphism and PsO susceptibility. However, they demonstrated the potential influence of these polymorphisms on clinical presentation and the clinical course of the disease in which the presence of C allele was more common among early onset of PsO.[25]

Comparing genotypes and allelic distribution between the PsA group to the control group revealed no statistically significant difference between the two groups (*p*> 0.05). However, we found an association between genotypes of TLR-9 SNP (rs5743836) and dactylitis and morning stiffness of peripheral joint in the PsA group (*p*>0.022).

In the study done by Oliveira-Toré et al., 2019, they found that TLR-9 rs5743836∗C is a susceptibility factor to PsA and, rs5743836∗T/C and rs5743836∗C/C genotypes are even greater risk factor for patients with PsA.[12] However, as we mentioned before there was a limitation in their study, as they compared the PsA group to a control group but not to a PsO group. Their study included a different ethnicity, Brazilian people, with a larger number of cases and used the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) technique.

In addition, our results showed an association between genotypes of TLR-9 SNP (rs5743836) and PASI score in PsO group (p< 0.05). This agreed with finding of a study by Kaeley et al., 2014, who reported that dactylitis is associated with more erosive forms of PsA.[30]

In 2011, Carvalho and his colleagues showed that the C allele of the single nucleotide polymorphism rs5743836 (T1237C) in TLR-9, displayed minor allele frequencies ranging from 0.02 to 0.38 across distinct ethnicities, predisposes to non-Hodgkin and Hodgkin lymphoma, as well as to several autoimmune and chronic inflammatory diseases, including asthma and Crohn's disease.[11.]

In conclusion, our results suggested an association between Toll-like receptor-2 rs5743708 and TLR-9 rs5743836 gene polymorphisms and susceptibility to PsO. However, they are not related to increase susceptibility to PsA in this cohort of Egyptian patients.

Author Contributions

SSK; Study design, Design of Data analysis, Manuscript review. NMA; Sample processing, Manuscript preparation. BME; Sample collection (Psoriatic arthritis). SAG; Sample collection (Psoriasis). NEA; Manuscript preparation

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of Faculty of Medicine (for Girls), Al- Azhar University (Approval number: 2018122001, dated June 2021).

Informed consent

An informed consent was obtained from each study participant.

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