Association between inducible nitric oxide synthase-954-G>C and Ex16+14-C>T gene polymorphisms and susceptibility to psoriasis and psoriatic arthritis

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Background
Psoriasis is a prevalent disorder of primarily skin and joint affection with a well-known genetic background and a sophisticated pathogenesis. The inducible nitric oxide synthase (iNOS) gene polymorphisms are unexplored areas of research when it comes to psoriasis.

Objectives
The aim of the study was to investigate the probable link between iNOS gene polymorphisms (-954 G/C and Ex 16+14C/T) and susceptibility to psoriasis and psoriatic arthritis (PsA).

Patients and methods
We included three groups of participants: 100 participants each of psoriasis, PsA and healthy controls. Genetic polymorphism analysis was performed utilizing the PCR with the restriction fragment length polymorphism method.

Results
Genetic analysis of iNOS polymorphism at Ex 16+14C/T revealed significantly increased CT genotype frequency and significantly lower CC genotype frequency in psoriasis (P=0.0011, 0.003, respectively) and PsA patients (P=0.001, $P<0.0001$, respectively) in comparison to controls. Genetic analysis of iNOS polymorphism at –954 G/C revealed insignificant difference in genotype distribution between psoriasis patients and controls, whereas significantly increased GC genotype frequency (P=0.038) and significantly decreased GG genotype frequency (P=0.038) were detected in PsA patients versus healthy controls.

Conclusions
iNOS polymorphism at Ex 16+14C/T, particularly the CT genotype, is associated with psoriasis in Egyptians, whereas PsA is associated with polymorphism at Ex 16 +14 and –954G/C.

Keywords:
954-G>, C, Ex16+14-C>, T, inducible nitric oxide synthase, nitric oxide, genetic polymorphism, psoriasis, psoriatic arthritis

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Introduction

With a complex genetic background and interplay of plenty of factors, psoriasis is a well-known multisystem inflammatory disorder. Predominant cutaneous and joint affection is a hallmark [1]. About 70% of disease susceptibility is attributable to genetic factors, with the remaining 30% influenced by environmental factors. Variable candidate genes that may be involved in the etiopathogenesis of psoriasis were outlined by genome-wide association studies. These encompass polymorphisms in genes that control interleukins (IL) 12, 17, and 23, among other factors [2].

Nitric oxide (NO) is a mediator that was widely investigated owing to its significant contribution to human well-being and physiology [3]. As multifunctional signaling molecule, it has been suggested as a potential candidate in the etiopathology of psoriasis as a powerful trigger of keratinocyte differentiation and proliferation [4].

Isoforms of nitric oxide synthase (NOS) have been named as neuronal (nNOS or NOS1), inducible (iNOS) or NOS2 and endothelial (eNOS or NOS3) [5]. Nitric oxide synthase 2 (NOS2) or iNOS is the one that is implicated in the innate immune response. Expression of iNOS mRNA is higher in psoriatic plaques than in the uninvolved skin according to Sirsjö *et al*. [6]. The iNOS can trigger the release of NO in tremendous quantities and can be expressed in

keratinocytes following stimulation by multiple mediators: IFN-γ, TNF-α, and IL-Iβ as examples [7].

In the present work, we try to investigate the probable relation between iNOS gene polymorphisms (-954 G/C and Ex 16+14C/T) and susceptibility to psoriasis and psoriatic arthritis (PsA).

Patients and methods

This case–control study was completed on 300 biologically unrelated patients of both sexes and age more than or equal to 18 years, selected from those attending the outpatient clinic of both Dermatology, Venereology and Andrology and Rheumatology and Rehabilitation during the period from February 2021 to February 2022. They were categorized into three groups: group I included 100 patients clinically diagnosed with different types of psoriasis (41 males and 59 females with a mean age of 43.5±15 years), group II with 100 patients with a confirmed diagnosis of PsA based on the Classification Criteria for Psoriatic Arthritis (CASPAR) [8] (40 males and 60 females with mean age 45.9±8.5 years), and group III with apparently healthy, psoriasis and PsA free, age-matched (P=0.123) and sex-matched (P=0.291) matched controls (50 males and 50 females with a mean age of 42.3±12.9 years).

Exclusion criteria included patients with any chronic dermatosis other than psoriasis, malignancy, chronic infection, or those on medications (including antipsoriatic/anti-inflammatory drugs) for the last 3 months, and pregnant or lactating females. Moreover, patients with all other forms of inflammatory arthritis including connective tissue disorders were dismissed from the PsA group.

The study scheme was affirmed by the local ethics committee in line with the percepts of Helsinki Declaration (MS: 6-7-2020). Informed consents were obtained from each participant. A comprehensive medical history was obtained from each participant followed by a thorough general examination including blood pressure records and BMI measurement using the 'weight in kilograms/height in m^2' formula. For psoriatic patients, a complete cutaneous examination was performed including the disease severity assessment using Psoriatic Area and Severity Index [9], while PsA patients received a complete musculoskeletal examination including the administration of the Disease Activity Index for Psoriatic Arthritis (DAPsA) [10] for the evaluation of PsA disease activity. The DAPsA score is calculated using the count of swollen and tender joints, patient evaluation of pain and disease activity, and C-reactive protein (CRP) level. Routine radiography was performed in PsA patients to evaluate peripheral joint affection. MRI was done for the detection of cervical spondylitis and sacroiliac changes. Erythrocyte sedimentation rate (ESR) was recorded for each participant.

Molecular study of gene variations: PCR-restriction fragment length polymorphism for the detection of inducible nitric oxide synthase gene polymorphism

Each participant had a venous blood sample (2 ml) drawn on EDTA. Each participant's blood sample was stored at −80°C in Eppendorf for additional preprocessing.

Genotyping of the polymorphisms −954 G/C (rs1800482) and Ex 16+14C/T (rs2297518) was performed using PCR-based restriction fragment length polymorphism in the following steps: (a) extracting DNA, (b) amplification of genomic DNA, and (c) digestion by the restriction enzymes BsaI and MluCI.

DNA extraction

The GF-1 blood DNA extraction kit, Catalog No. GF-BD-100, was used to extract DNA from a 100 µl blood sample (Vivantis Technologies, Malaysia), as provided by the manufacturing company. Elution of DNA was accomplished using 50 different elution buffers. A NanoDrop Spectrophotometer 2000 was implemented to assess the concentration of extracted DNA (Thermo-Fisher Scientific, Wilmington, Delaware, USA). Wave lengths of 260 and 280 nm were measured [11]. The optical density (OD) ratio at 260 and 280 nm was used to estimate DNA purity. The OD260/OD280 ratio of pure DNA preparations is 1.7–2.0. The ratio is 1.7 when contaminated with protein or phenol, but more than 2.0 when contaminated with RNA. The extracted DNA was kept at −80°C until it was further processed.

Genomic DNA amplification

Primers of iNOS were used for DNA amplification in 50 µl reactions per sample. Forward 5'-CATATGT ATGGGAATACTGTATTTCAG-3' and reverse 5'-TCTGAACTA GTCACTTGAGG-3' primers were used for iNOS −954 G/C genotyping. For iNOS Ex16+14C/T genotyping, the following primer was used: forward 5'-CATATGTAAA CCAACTTCCGTG-3' and reverse 5'-GCAGGG CTAGGAGTAGGAC-3'. The iNOS −954 G/C

and iNOS. The amplified PCR products for Ex16 +14C/T genes were 573 and 219 bp, respectively.

Veriti Thermal Cycler (Applied Biosystems, UK) was used for amplification. The reaction mixture included 25 µl of Easy taq PCR SuperMix (Transgen Biotech, China), 1 µl of FP (Forward Primer), 1 µl of reverse primer, 5 µl of DNA, and 50 µl of nuclease-free water. The PCR conditions follow the next steps: 5 min of initial denaturation at 95°C, 35 cycles (denaturation at 95°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 1 min), and 5 min of final extension at 72°C. To check the PCR products at 573 219 bp, PCR products (10 l) and a 100 base pair ladder (5 l) were resolved in a 3% agarose gel stained with 0.3 µg/ml ethidium bromide.

The entire procedure was adapted from the previously standardized protocol of Shen *et al.* [11].

Digestion by BsaI and MluCI restriction enzymes (New England Biolabs)

Digestion of the iNOS PCR gene product was performed in a total volume of 50 µl by combining: 1 µl of PCR products+1 µl BsaI restriction enzyme (1 U)+5 µl 10× NEBuffer+up to 50 µl nuclease-free water. The digestion mixtures were kept overnight at 37°C before being inactivated for 20 min at 80°C. The same procedure was followed for the MluCI restriction enzyme.

On a 3% agarose gel, DNA fragments were separated and stained with 0.3 µg/ml ethidium bromide. The PCR products after restriction enzyme cutting were as follows: 437 136 for iNOS −954 polymorphic alleles. There were 175, 44 polymorphic alleles for iNOS Ex 16+14. The bands (predigestion and postdigestion) were imaged with an 8-megapixel digital camera and visualized with a ultraviolet transilluminator (254 nm). Computer software was used to analyze the image (Alpha InoTech Gel Documentation System).

Data processing and statistical analysis

After collection, revision, coding, and tabulation of the data, it was uploaded to a PC using IBM Corp.'s Statistical Package for the Social Sciences; IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, New York, USA). The standard data distribution was assessed using Kolmogorov–Smirnov test. Descriptive statistics were expressed as mean and SD for numerical data, and as frequency and percentage of nonnumerical data. While analysis of variance test, post-hoc analysis, independent *t* test, and χ^2 test were utilized for analytical statistics. A *P* value of 0.05 or less marks the level of significance.

Results

Demographic data

Among those with psoriasis there were 41 males and 59 females with a mean age of 43.5±15 years, PsA patients were 40 males and 60 females with a mean age of 45.9 ±8.5 years, while controls were 50 males and 50 females with a mean age of 42.3±12.9 years ensuring intergroup age (P=0.123) and sex (P=0.291) matching.

Clinical characteristics of the studied patients

Regarding clinical data, psoriasis patients had a mean systolic blood pressure (SBP) of 134±14 mmHg, mean diastolic blood pressure (DBP) of 77±9 mmHg, and a mean BMI of 28.4±4.4 kg/m^2. PsA patients had a mean SBP of 133±21 mmHg and mean DBP of 78 ±9 mmHg, with a mean BMI of 29.3±3.4 kg/m^2. Controls had a mean SBP of 129±24 mmHg, mean DBP of 75±10 mmHg, and a mean BMI of 27±3.2 kg/m^2. Insignificant difference was detected between the studied groups regarding either SBP (P=0.198), DBP (P=0.847), or BMI (P=0.347). Clinical data of the studied groups are shown in Table 1.

Analysis of inducible nitric oxide synthase polymorphism at Ex 16+14C/T

Genetic analysis of iNOS polymorphism at Ex 16 +14C/T revealed significantly increased CT genotype frequency (P=0.0011) and significantly decreased CC genotype frequency (P=0.0003) in psoriasis patients compared with controls. Allelic frequency analysis of iNOS polymorphism at Ex 16 +14C/T revealed that regarding second allele, there was significantly increased frequency of T allele and significantly decreased frequency of C allele in psoriasis patients compared with controls (P=0.001).

Genetic analysis of iNOS polymorphism at Ex 16 +14C/T revealed significantly increased CT genotype frequency (P=0.001) and significantly decreased CC genotype frequency (P<0.0001) in PsA patients compared with controls. Allelic frequency analysis of iNOS polymorphism at Ex 16 +14C/T revealed that regarding second allele, there was significantly higher frequency of T allele and significantly lower frequency of C allele in PsA patients in comparison to controls (P=0.001).

Analysis of inducible nitric oxide synthase polymorphism at −954 G/C

Genetic analysis of iNOS polymorphism at −954 G/C revealed no significant difference in genotype distribution in psoriasis patients compared with controls. Allelic frequency analysis of iNOS polymorphism at −954 G/C revealed insignificant

Table 1 Clinical characteristics of psoriasis and psoriatic arthritis patients

Psoriasis group	Psoriatic arthritis group		
Onset of disease			
Gradual			
50	Duration of stiffness (min)	Mean±SD	19.8 ±23
Indolent			
17			
Sudden		Range	1–60
33			
Course			
Acute		Mean±SD	11.8±7
23	Tender joints number		
Progressive			
23			
Stationary			
44			
Recurrent			
3		Range	2–25
Regressive			
7			
Duration (years)			
Mean±SD			
6.1±6.4	Swollen joints number	Mean±SD	8.1±6
Range			
1–25		Range	1–20
Family history of psoriasis			
Positive			
17	Axial joints affection	Positive	44
Negative			
83		Negative	56
PASI severity			
Mild			
37	Peripheral joints affection	Positive	87
Moderate			
60		Negative	13
Severe			
3			
Scalp affection			
Positive			
46	Sacroiliac joint affection	Positive	44
Negative			
54		Negative	56
Nail affection			
Positive			
45	Radiograph manifestations	Positive	83
Negative			
55		Negative	17
Type of psoriasis			
Plaque			
79	MRI manifestations	Cervical spondylitis	20
Erythrodermic			
3		Sacroiliac erosions	22
Guttate			
18		Non	58
Distribution of lesions			
Localized			
27			
Symmetrical			
10		Mean±SD	4.9 ±2.2
Discrete			
34	Joint pain score		
Diffuse			
26		Range	1–8
Generalized			
3			
Symptoms			
Non			
50			
Itching			
47	Disease activity score	Mean±SD	4.9 ±2.1
Fever and burning			
3		Range	1–8
Arthritis			
Present			
34		Mean±SD	39.68 ±20.1
Absent			
66	DAPsA score	Range	10–80
Disease activity	Low activity		12
		Moderate activity	24
		High activity	64
	ESR (mm/h)	Mean±SD	37.4 ±15
		Range	8–60
	CRP (mg/l)	Mean±SD	10±3.7
		Range	2–20

CRP, C-reactive protein; DAPsA, disease activity of psoriatic arthritis; ESR, erythrocyte sedimentation rate; PASI, psoriasis activity severity score.

difference in allelic distribution in psoriasis patients compared with controls (P=0.102).

Genetic analysis of iNOS polymorphism at -954 G/C revealed significantly higher frequency of GC genotype (P=0.038) and significantly lower frequency of GG genotype (P=0.038) in PsA patients compared with controls. Allelic frequency analysis of iNOS polymorphism at -954 G/C revealed that regarding second allele, there was significantly increased C allele frequency and significantly decreased G allele frequency in PsA patients compared with controls (P=0.037).

Table 2 Genotype frequency of inducible nitric oxide synthase polymorphism at Ex 16+14C/T and –954 G/C in psoriasis and psoriatic arthritis

Ex 16 +14C/T					
Genotype	Psoriasis	Controls	OR	95% CI	*P* value
CC	34	60	0.343	0.19	0.0003 (HS)
CT	54	31	2.61	1.465	0.0011 (HS)
TT	12	9	1.378	0.553	0.49 (NS)
	Psoriatic arthritis				
CC	30	60	0.285	0.159	<0.0001(HS)
CT	59	31	3.20	1.7	0.001 (HS)
TT	11	9	1.24	0.49	0.63 (NS)
-954 G/C					
Genotype	Psoriasis	Controls	OR	95% CI	*P* value
GG	70	80	0.58	0.30	0.104 (NS)
GC	30	20	1.71	0.89	0.104 (NS)
CC	0	0			
	Psoriatic arthritis				
GG	67	80	0.5	0.26	0.038 (S)
GC	33	20	1.97	1.03	0.038 (S)
CC	0	0			

CI, confidence interval; HS, highly significant; NS, nonsignificant; OR, odds ratio; S, significant. Using χ^2 test, *P* value less than or equal to 0.05 is significant.

Genotype frequency of iNOS polymorphism at Ex 16 +14C/T and –954 G/C in psoriasis and PsA are clarified in Table 2.

Ex 16+14C/T inducible nitric oxide synthase polymorphism in relevance to clinical and demographic data

iNOS polymorphism at Ex 16+14C/T in psoriasis patients revealed that the CT genotype is significantly associated with longer duration of disease, stationary course, scalp and nail affection, plaque psoriasis, discrete and diffuse distribution of disease, and arthritis. CC genotypes is significantly linked to lower SBP and DBP and localized distribution of the disease, while the TT genotype is associated with higher BMI. Itching was significantly presented with both CC and CT genotypes (Table 3).

iNOS polymorphism at Ex 16+14C/T in PsA patients revealed that the number of tender and swollen joints was significantly higher with the TT genotype followed by the CT genotype and least with the CC genotype. ESR and CRP were significantly higher with the TT genotype followed by the CT genotype and least with the CC genotype. Scores of joint pain, disease activity, and DAPsA score were significantly higher with the TT genotype followed by the CT genotype and least with the CC genotype. Peripheral and sacroiliac joint affection were significantly associated with the CT genotype. Moreover, cervical spondylitis diagnosed with MRI was significantly associated with the CT genotype (Table 4).

-954 G/C inducible nitric oxide synthase polymorphism in relevance to clinical and demographic data

iNOS polymorphism at –954 G/C in psoriasis patients revealed that the GG genotype was significantly associated with sudden onset of the disease, acute and progressive course of disease, mild disease severity, and localized distribution of the disease. Nail affection, arthritis, and longer duration of the disease are associated with the GC genotype (Table 5).

iNOS polymorphism at –954 G/C in PsA patients revealed that the GC genotype had significantly longer duration of stiffness, increased number of tender and swollen joint affection, higher level of ESR and CRP, higher score of joint pain, and DAPsA scores. The GC genotype was significantly associated with axial joint affection, sacroiliac joint affection, and erosions. However, the GG genotype was significantly associated with peripheral joint affection (Table 6).

Discussion

In genes, the transcription level regulates the gene expression. Variability in the promotor region alters the gene product within the cell, whereas variability in the coding regions can trigger modifications in the action of the protein product and consequently may be responsible for disease susceptibility [12]. This is also typical for iNOS gene expression [13].

The iNOS-954-G/C promotor polymorphism modifies the iNOS level in cells, while iNOS-Ex16

Table 3 Analysis of inducible nitric oxide synthase polymorphism at Ex 16+14C/T in relation to demographic and clinical characteristics in psoriasis patients

	CC	CT	TT	*P* value	Post-hoc analysis
Age (years)					
Mean±SD	43.17±17.7	43.61±12.4	43.67±18.9	0.990a (NS)	–
Duration of disease (years)					
Mean±SD	3.66±5.7	8.12±6.6	3.54±3.9	0.002a (S)	(1) 0.001 (2) 0.953 (3) 0.020
SBP (mmHg)					
Mean±SD	125.79±11.1	139.1±13.2	135.56±18.8	0.005a (S)	(1) 0.001 (2) 0.080 (3) 0.491
DBP (mmHg)					
Mean±SD	72.63±7.5	80±9	75.56±11	0.018a (S)	(1) 0.006 (2) 0.420 (3) 0.189
BMI					
Mean±SD	26.97±4.6	28.83±3.9	30.12±4.4	0.044a (S)	(1) 0.049 (2) 0.028 (3) 0.329
Sex					
Male (*n*)	16	23	2	0.173b (NS)	–
Female (*n*)	18	31	10		
Family history of psoriasis					
Positive (*n*)	2	12	3	0.102b (NS)	–
Negative (*n*)	32	54	12		
Onset of disease					
Gradual (*n*)	18	27	5	0.066b (NS)	–
Indolent (*n*)	2	14	1		
Sudden (*n*)	14	13	6		
Course					
Acute (*n*)	5	12	6	0.001b (S)	–
Progressive (*n*)	11	7	5		
Stationary (*n*)	11	32	1		
Recurrent (*n*)	0	3	0		
Regressive (*n*)	7	0	0		
PASI severity					
Mild (*n*)	29	5	3	0.001b (S)	–
Moderate (*n*)	5	49	6		
Severe (*n*)	0	0	3		
Scalp affection					
Positive (*n*)	11	26	9	0.035b (S)	
Negative (*n*)	23	28	3		
Nail affection					
Positive (*n*)	5	36	4	0.001b (S)	
Negative (*n*)	29	18	8		
Type of psoriasis					
Plaque (*n*)	27	45	7	0.001b (S)	
Erythrodermic (*n*)	0	0	3		
Guttate (*n*)	7	9	2		
Distribution of lesions					
Localized (*n*)	20	4	3	0.001b (S)	
Symmetrical (*n*)	6	3	1		
Discrete (*n*)	7	24	3		
Diffuse (*n*)	1	23	2		
Generalized (*n*)	0	0	3		
Symptoms					
Non (*n*)	20	25	5	0.001b (HS)	
Itching (*n*)	14	29	4		
Fever and burning (*n*)	0	0	3		
Arthritis					
Present (*n*)	0	32	2	0.001b (HS)	
Absent (*n*)	34	22	10		

(1): CC versus CT, (2) CC versus TT, (3) CT versus TT. DBP, diastolic blood pressure; HS, highly significant; NS, nonsignificant; PASI, Psoriatic Area and Severity Index; S, significant; SBP, systolic blood pressure. [a]One-way analysis of variance test. [b]χ^2 test. *P* value less than or equal to 0.05 is significant.

Table 4 Analysis of inducible nitric oxide synthase polymorphism at Ex 16+14C/T in relation to demographic and clinical characteristics in psoriatic arthritis patients

	CC	CT	TT	*P* value	Post-hoc analysis
Age					
Mean±SD	45.4±7.9	46.4±8.8	44.3±9.4	0.703a (NS)	–
SBP					
Mean±SD	136.3±10.1	133.7±25.4	125.8±12	0.597a (NS)	–
DBP					
Mean±SD	77.2±3.1	79.5±9.7	73.3±9.7	0.243a (NS)	–
Duration of stiffness					
Mean±SD	23±12	18.3±10.6	19.1±10.2	0.663a (NS)	–
Number of tender joints					
Mean±SD	3.93±1.8	13.3±4.7	25±4.5	0.001a (HS)	(1) 0.001 (2) 0.001 (3) 0.001
Number of swollen joints					
Mean±SD	1.63±0.7	9.2±4.3	20±6.9	0.001a (HS)	(1) 0.001 (2) 0.001 (3) 0.001
ESR					
Mean±SD	19.2±8	42.7±9.2	58.2±4	0.001a (HS)	(1) 0.001 (2) 0.001 (3) 0.001
CRP					
Mean±SD	5.7±2.2	11.1±1.6	15.9±2.4	0.001a (HS)	(1) 0.001 (2) 0.001 (3) 0.001
Joint pain score					
Mean±SD	2.33±1.2	5.82±1.4	7.2±2.4	0.001a (HS)	(1) 0.001 (2) 0.001 (3) 0.001
DAPsA score					
Mean±SD	15.87±5.2	45.2±12.6	75.3±2.7	0.001a (HS)	(1) 0.001 (2) 0.001 (3) 0.001
Disease activity					
Low activity	12	0	0	0.001b (HS)	
Moderate activity	18	6	0		
High activity	0	53	11		
Sex					
Male (*n*)	11	22	7	0.237b (NS)	
Female (*n*)	19	37	4		
Axial joint affection					
Positive (*n*)	13	24	7	0.370b (NS)	
Negative (*n*)	17	35	4		
Peripheral joint affection					
Positive (*n*)	20	57	10	0.001b (S)	
Negative (*n*)	10	2	1		
Sacroiliac joint affection					
Positive (*n*)	13	20	11	0.001b (S)	
Negative (*n*)	17	39	0		
Radiograph manifestations					
Positive (*n*)	24	50	9	0.848b (NS)	
Negative (*n*)	6	9	2		
MRI manifestations					
Cervical spondylitis (*n*)	0	20	0	0.001b (S)	
Sacroiliac erosions (*n*)	0	11	11		

[a]One-way analysis of variance test. [b]χ^2 test. (1) CC versus CT, (2) CC versus TT, (3) CT versus TT. CRP, C-reactive protein; DAPsA, disease activity of psoriatic arthritis; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; HS: highly significant; NS: nonsignificant; S: significant; SBP: systolic blood pressure. *P* value less than or equal to 0.05 is significant.

+14-C/T coding polymorphism causes amino acid derangement resulting in changed protein product [14]. This could be relevant in psoriasis, as iNOS was suggested to increase in psoriatic lesional skin where it could participate crucially in vascular events happening in the psoriatic skin [6]. Meki and Al-Shobaili [15] reported increased NO levels in psoriatic patients as an indicator of oxidative stress among others that was connected to disease severity.

The current work analysis of iNOS polymorphism at Ex 16+14C/T revealed significantly increased CT genotype frequency (P=0.001) and significantly lower CC genotype frequency (P<0.001) in psoriasis patients than comparable controls. The second T allele

Table 5 Analysis of inducible nitric oxide synthase polymorphism at –954 G/C in relation to demographic and clinical characteristics in psoriasis patients

	GG (*N*)	GC (*N*)	*P* value
Onset of disease			
Gradual	30	20	0.001a (S)
Indolent	9	8	
Sudden	31	2	
Course			
Acute	21	2	0.005a (S)
Progressive	17	6	
Stationary	24	20	
Recurrent	1	2	
Regressive	7	0	
Family history of psoriasis			
Positive	7	10	0.074a (NS)
Negative	63	20	
PASI severity			
Mild	35	2	0.001a (S)
Moderate	33	27	
Severe	2	1	
Scalp affection			
Positive	28	42	0.066a (NS)
Negative	18	12	
Nail affection			
Positive	20	25	0.001a (S)
Negative	50	5	
Type of psoriasis			
Plaque	53	26	0.395a (NS)
Erythrodermic	2	1	
Guttate	15	3	
Distribution of lesions			
Localized	26	1	0.006a (S)
Symmetrical	8	2	
Discrete	19	15	
Diffuse	15	11	
Generalized	2	1	
Symptoms			
Non	38	12	0.442a (NS)
Itching	30	17	
Fever and burning	2	1	
Arthritis			
Present	9	25	0.001a (S)
Absent	61	5	
Age			
Mean±SD	42.67±15.7	45.33±13.4	0.990b (NS)
Duration of disease			
Mean±SD	4.38±2.7	9.97±4.1	0.001b (S)
SBP			
Mean±SD	134.9±14.7	133.2±14.7	0.696b (NS)
DBP			
Mean±SD	77.7±8.8	75.29±10.7	0.368b (NS)
BMI			
Mean±SD	28.2±4.6	28.7±3.6	0.577b (NS)

[a]Using χ^2 test. [b]Using independent *t* test. DBP, diastolic blood pressure; HS, highly significant; NS, nonsignificant; PASI, Psoriatic Area and Severity Index; S, significant; SBP, systolic blood pressure. *P* value less than or equal to 0.05 is significant.

Table 6 Analysis of inducible nitric oxide synthase polymorphism at –954 G/C in relation to clinical and demographic characteristics in psoriatic arthritis patients

	GG (*N*)	GC (*N*)	*P* value
Sex			
Male	32	9	0.143a (NS)
Female	38	21	
Age			
Mean±SD	46.85±8.6	43.9±8.2	0.108a (NS)
SBP			
Mean±SD	135.4±14.7	130.2±29.8	0.374a (NS)
DBP			
Mean±SD	77.8±8	79±10.2	0.464a (NS)
Duration of stiffness			
Mean±SD	14.78±6.9	30±11.2	0.002a (S)
Number of tender joints			
Mean±SD	7.69±4.2	20.1±4.6	0.001a (S)
Number of swollen joints			
Mean±SD	4.51±1.4	15.5±4.1	0.001a (S)
ESR			
Mean±SD	29.54±12.3	53.33±4.8	0.001a (S)
CRP			
Mean±SD	8.22±2.8	13.61±2.3	0.001a (S)
Joint pain score			
Mean±SD	3.81±1.8	7.21±0.69	0.001a (S)
DAPsA score			
Mean±SD	28.06±13.2	63.27±11.1	0.001a (S)
Disease activity			
Low activity	12	0	0.001b (HS)
Moderate activity	24	0	
High activity	31	33	
Axial joint affection			
Positive	19	25	0.001b (S)
Negative	48	8	
Peripheral joint affection			
Positive	55	32	0.037b (S)
Negative	12	1	
Sacroiliac joint affection			
Positive	17	27	0.001b (S)
Negative	50	6	
Radiograph manifestations			
Positive	54	29	0.362b (NS)
Negative	13	4	
MRI manifestations			
Cervical spondylitis	13	7	0.001b (S)
Sacroiliac erosions	0	22	
Non	54	4	

[a]Using independent *t* test. [b]Using χ^2 test. CRP, C-reactive protein; DAPsA, disease activity of psoriatic arthritis; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; HS, highly significant; NS, nonsignificant; S, significant; SBP, systolic blood pressure. *P* value less than or equal to 0.05 is significant.

seems to be linked to increased risk of psoriasis and PsA. The CT genotype was associated with longer disease duration, while the TT genotype was associated with higher BMI. The CC genotype was associated with lower SBP and DBP.

Evidence of association between hypertension and psoriasis is strongly growing [16], not to mention the interrelation between severity of psoriasis and poor control of blood pressure [17]. As a potent vasodilator and crucial player in endothelial oxidative stress events, NO is known to contribute to hypertension. Inhibition of iNOS has been proposed as a potential target for the treatment of hypertension [18].

Obesity is an identifiable risk factor for the initiation and amplification of psoriatic inflammation, based on several research findings [19]. Expansion of adipose tissue – which in fact act as an endocrinal organ – participates in the efflux of variable mediators involved in both immunity and inflammation, resistin, and leptin as examples [20].

Analysis of iNOS polymorphism at –954 G/C revealed no significant differences between psoriatic patients and controls, but a significantly higher GC genotypic frequency (P=0.038) and significantly lower GG genotypic frequency (P=0.038) was reported in the PsA patient group in the current work compared with the control group. The second C allele at –954G/C seems to be associated with susceptibility to PsA. The GC genotype is associated with longer duration of psoriasis, while GG is linked to the acute progressive disease.

Large amounts of NO are proved to be synthesized locally at sites of autoinflammation by iNOS; a cytotoxic role has been suggested. Later, autoinflammation was exaggerated by the inhibition of iNOS, so an immunoregulatory role is proposed instead [21]. This goes in line with the results of the current work. This may pave a road in explaining the potential role of iNOS in psoriasis and PsA etiopathogenesis; hence, the genetic polymorphism which may explain the genetic susceptibility of both conditions.

Duan *et al.* [22] did not report a significant relation between psoriasis and iNOS –954G/C polymorphisms in the Han Chinese population in line with our findings despite different studied ethnicities, and similar to results obtained by Coto-Segura *et al.* [23] who studied Spanish patients, but contradictory to the Turkish study that was first to investigate iNOS –954G/C polymorphisms in psoriasis and reported the implication of the gene polymorphisms in the susceptibility for psoriasis. The ethnic variation explains it all and needs further large-scale research. To the best of our knowledge, we are the first to investigate the iNOS Ex 16 +14C/T promotor region polymorphisms and –954G/C coding region polymorphisms in PsA, despite other iNOS promotor region polymorphisms had been investigated in other types of arthritis, namely rheumatoid arthritis (RA) [24]. NO is among the potential mechanisms causing bone erosion in RA as the synovial fluid of RA patients showed increased levels of iNOS [24].

Previous research has demonstrated that 277 A/G and 1026 G/T polymorphisms in the iNOS promoter region enhanced the possibility of having RA [24,25].

Our results revealed – upon analysis of iNOS polymorphisms – that the TT and GC genotypes correlate with PsA severity as evaluated by the DAPsA score.

Polymorphisms of iNOS had been widely investigated in autoimmune diseases. Polymorphism at iNOS-954 G/C and Ex 16+14C/T has been researched in vitiligo [26,27]. Polymorphisms (CCTTT)n microsatellite and 954 G/C has been linked to the emergence of systemic lupus erythematosus [28].

The iNOS gene polymorphisms in patients with psoriasis and PsA could open the door for new diagnostic markers and therapeutic targets. Further larger studies on different ethnicities, and evaluation of the activity of iNOS in psoriasis and PsA – in particular – may allow for the generalization of the results of the current work.

Conclusion

In the context of the strong genetic predisposition of psoriasis and PsA, the iNOS gene polymorphism may be a new step in the identification of the accused genes. We concluded that iNOS polymorphism at Ex 16 +14C/T, particularly the CT genotype, is associated with psoriasis in a sample of Egyptians, while polymorphism at both Ex 16+14 and –954G/C is associated with PsA.

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Authors' contribution: the manuscript has been read and approved by all authors. Each author believes that the manuscript represents honest work. Ahmed M. Hamed, Sura M. Naji, Mayada E. Youssef, and Ghada M. Shams performed the research. Ahmed M. Hamed and Ghada M. Shams designed the research study. Hend E. Nasr, Ghada M. Shams, and Ahmed M. Hamed contributed essential reagents. Hend E. Nasr,

Mayada E. Youssef, and Sura M. Naji analyzed the data.

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There are no conflicts of interest.

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