Original Article

Assessment of the Possible Role of FOXP3 Gene (rs3761548) Polymorphism in Psoriasis Vulgaris Susceptibility and Pathogenesis: Egyptian Study

Abstract

Background: Psoriasis is an autoimmune-related chronic inflammatory skin disorder. Psoriasis vulgaris (PV) is the most common form of psoriasis. T regulatory cells (Tregs) are typically considered inhibitors of autoimmune responses. FOXP3 is a master control transcription factor for development and function of Tregs. FOXP3 gene polymorphism changes FOXP3 protein function and quantity leading to Tregs dysfunction that subsequently may be related to PV pathogenesis. **Objective:** The objective of the present study was to evaluate the possible role of FOXP3 gene (rs3761548) polymorphism in PV pathogenesis. **Materials and Methods:** One hundred sixty subjects were included in the present study (80 PV patients and 80 well-matched healthy controls). All participants were evaluated by detailed history, general examination, dermatological examination, and psoriasis area and severity index (PASI) score. The detection of FOXP3 gene (rs3761548) polymorphism in patients and controls by PCR-restriction fragment length polymorphism technique was done. **Results:** There was statistically significant increase in CC genotype and C allele in patients compared to controls, whereas there were non-significant differences in AA and AC genotypes. However, there were non-significant associations between genotype distribution and each of age, sex, family history, PASI score, hair affection, nail affection, hypertension, diabetes mellitus, and body mass index. **Conclusion:** FOXP3 gene (rs3761548) polymorphism may increase susceptibility of PV and share in its pathogenesis as it leads to changes in FOXP3 protein function and quantity that subsequently affect T-regs functions. Further investigations for the role of other FOXP3 genes polymorphisms in psoriasis pathogenesis and their effects on the treatment response in psoriasis patients are strongly recommended.

Keywords: *FOXP3, psoriasis, regulatory T cells*

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Introduction

Psoriasis is an immune-mediated chronic inflammatory skin disorder, and psoriasis vulgaris (PV) is its most common form.[1] It affects about 2% of the world population. Psoriasis is a complex multifactorial condition related to a combination of genetic, environmental, and immunological factors. T regulatory cells (Tregs) are essential for immune homeostasis by virtue of their ability to suppress the function of other lymphocytes, so suppressing immune responses, inflammation, and tissue destruction. The stable expression of the T-reg master transcription factor, fork head box 3 (Foxp3), is crucial for T-reg function. FOXP3 gene on the p arm of the X-chromosome (specifically, Xp11.23) encodes FOXP3 protein synthesis.[2]

In psoriasis, the expression of FOXP3 protein was found to be decreased or aberrant. This may be due to FOXP3 gene polymorphism. FOXP3 protein abnormality leads to functional defect of Tregs that may play a role in psoriasis pathogenesis.[3,4]

Psoriasis is increasingly recognized as a multisystem inflammatory condition associated with a range of co-morbid diseases including obesity, metabolic syndrome, cardiovascular disease, psoriatic arthritis, autoimmune diseases, psychiatric illness, liver disease, and sexual dysfunction.[5]

The aim of the present study was to evaluate the possible role of FOXP3 gene (rs3761548) polymorphism in psoriasis susceptibility and pathogenesis in Egyptian patients.

How to cite this article: Elsohafy MA, Elghzaly AA, Abdelsalam HM, Gaballah MA. Assessment of the Possible Role of FOXP3 Gene (rs3761548) Polymorphism in Psoriasis Vulgaris Susceptibility and Pathogenesis: Egyptian Study. Indian Dermatol Online J 2019;10:401-5.

Received: October, 2018. **Accepted:** November, 2018.

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Access this article online

Website: www.idoj.in

DOI: 10.4103/idoj.IDOJ_372_18

Quick Response Code:

Materials and Methods

This study was conducted in the Dermatology outpatient clinic, Department of Dermatology, Andrology and Sexual Transmitted Diseases, Faculty of Medicine, Mansoura University Hospital from July 2015 to December 2016. Eighty PV patients (patient group) and 80 age and sex matched apparently healthy individuals (control group) were included. The sample size was calculated using Epi info version 6.04. According to the statistical data, power of study was 80%, 95% confidence interval. Informed written consent was obtained from all enrolled patients and controls. Approval of Institution Review Board (IRB) in Mansoura Faculty of Medicine was obtained (number MS/15.06.78).

Patients with other skin diseases or with chronic diseases (other than hypertension and diabetes mellitus [DM]) such as pulmonary, hepatic, renal, hematological, neurologic, psychiatric disorders, and malignancy were excluded.

All participants were subjected to detailed history taking, full general examination [including body mass index (BMI) calculation (weight/height^2) and blood pressure measurement], full dermatological examination (including skin, nail, hair, and mucous membrane), and estimation of severity of PV according to psoriasis area and severity index (PASI) score.[6]According to the age of onset, psoriasis patients were divided into type I (early-onset; starts before the age of 40) and type II (late-onset; starts after the age of 40).[7]

Venous blood sample of 1 ml was taken from each patient and control to detect FOXP3 gene (rs3761548) polymorphism. Genomic DNA was extracted from peripheral blood mononuclear cells using Qiagen blood DNA extraction mini kit according to the manufacturer's instructions. DNA quantity and quality were checked.

PCR amplification for FOXP3

PCR was performed in a volume of 30 µL, with 1 µL (20 ng) of genomic DNA and 2 µL (10 pmol) of each of following primer: "F: 5-GCCCTTGTCTACTCCACGCCTCT-3" and "R: 5-CAGCCTTCGCCAATACACAGAGCC-C-3," 15 µL of polymerase enzyme, and 10 µL of distilled water H_2O. The parameters for PCR include an initial denaturing step at 98°C for 1 min, followed by 35 cycles of 98°C for 30 s, annealing for 30 s, and extension at 72°C for 1 min, and a final extension at 72°C for 7 min. To 30 µl PCR reaction system, 1 kb fragment of human genomic DNA was amplified by using 2× Taq PCR Master Mix.

PCR cycle set up

Load 5 µl PCR products to agarose gel for PCR detection.

Restriction fragment length polymorphism for rs3761548A/C

A 15 µL aliquot of PCR product will be digested with 1 µl restriction enzyme (PST I) at 37°C for 16 hours and then separated on a 2% agarose gel, PCR product length after cutting will be (Tm/°C) 487 bp/ 63°C.

Detection of FOXP3 gene polymorphism

For PCR reaction set-up, users only need to pipette an aliquot part of 2× Taq PCR Master Mix and dilute the Master Mix to 1× by adding templates, primers, and water up to the reaction volume. There are two types of this product: Master Mix with loading dye (blue) and Master Mix without loading dye (colorless). PCR products produced by using Master Mix with loading dye can be loaded directly without extra loading buffer, and we used this method in the current study.

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0 (IBM Corporation, Armonk, New York, USA). Qualitative data were described using number and percent. Quantitative data were described using mean and standard deviation or median and range. The P value <0.05 was considered to be statistically significant. The following tests were done: Chi-square test, binary logistic regression, Mann-Whitney test, and Hardy-Weinberg equation.

Results

Patients were 31 males (38.8%) and 49 females (61.2%), and their ages ranged between 22 and 68 years. There were non-significant differences between patients and controls regarding age, sex, and DM. However, hypertension and BMI were significantly increased in psoriatic patients. The PASI score of patients ranged from 0.3 to 18.3 [Table 1]. The PASI score was significantly increased in patients with late onset psoriasis (above forty) than those with early onset psoriasis (less than forty). In addition, PASI score showed significant increases in female patients, in hypertensive patients, and in diabetic patients. Finally, the PASI score showed a non-significant direct correlation with BMI [Table 2].

CC genotype and C allele were significantly higher in patients than controls. Moreover, CC genotype was significantly the highest genotype in patients than other genotypes, and the possibility of having PV is high in patient with this genotype than other genotypes [Table 3].

CC genotype is a significant predictor of PV, but, not AC and AA genotypes [Table 4]. There were non-significant associations between genotype distribution and each of age, sex, family history, PASI score, hair affection, nail affection, hypertension, DM, and BMI in patient group.

Discussion

In the current study, hypertension and obesity were significantly more prevalent in psoriatic patients than controls. El-Shahat *et al*.[8] and Al-Mutairi *et al*.[9] reported

Table 1: Demographic data and clinical criteria of patients and controls

	Patients *n*=80 *n* (%)	Controls *n*=80 *n* (%)	Test of significance	OR (95% CI)
Age				
>40	46 (57.5)	48 (60.0)	χ^2=0.1	1
≤40	34 (42.5)	32 (40.0)	*P*=0.7	1.1 (0.59-2.08)
Sex				
Male	31 (38.8)	34 (42.5)	χ^2=0.23	1
Female	49 (61.2)	46 (57.5)	*P*=0.6	1.17 (0.62-2.19)
Hypertension				
−ve	23 (28.8)	45 (56.3)	χ^2=12.4	1
+ve	57 (71.2)	35 (43.8)	*P*=0.004*	3.18 (1.65–6.14)
DM				
−ve	18 (22.5)	21 (26.2)	χ^2=0.31	1
+ve	62 (77.5)	59 (73.8)	*P*=0.58	1.22 (0.59–2.53)
BMI (KG/m^2) Mean±SD	35.02±2.5	33.89±1.4	*t*=3.52 *P*=0.01*	
PASI score Median (Min-Max)	4.4 (0.3–18.3)	-	-	-

χ^2=Chi-square test, *t*=student *t* test, DM=Diabetes mellitus, BMI=Body Mass Index, OR=Odds ratio, SD=Standard deviation; **P* is significant statistically if <0.05, PASI=Psoriasis area severity index

Table 2: Correlation of PASI score with age, sex, hypertension, DM, and BMI

Item	PASI	Test of significance
Age		
>40	5.4 (0.3–18.3)	*z*=3.8
≤40	1.8 (0.3–13.0)	*P*=0.001*
Sex		
Male	1.8 (0.3–14.4)	*z*=2.7
Female	4.9 (0.9–18.3)	*P*=0.006*
Hypertension		
+ve	5.4 (1.2–18.3)	*z*=2.36 *P*=0.02*
−ve	3.6 (0.3–16.1)	
DM		
+ve	6.6 (4.0–16.1)	*z*=3.4
−ve	2.8 (0.3–18.3)	*P*=0.001*
BMI (Kg/m^2)#	*r*=0.215	
	P=0.52	

z=Mann-Whitney U test; **P* value significant<0.05. #BMI continuous variables so relation was calculated using spearman correlation coefficient. DM=Diabetes mellitus, PASI=Psoriasis area severity index, BMI=Body Mass Index

that psoriatic patients had higher incidence of obesity, DM, and hypertension than controls. In addition, Love *et al*.[10] reported a significantly increased risk of DM, hypertension, and obesity in patients with psoriasis compared with controls even after adjustment for age, sex, race/ethnicity, smoking, and C-reactive protein levels. Moreover, Pietrzak *et al*.[11] found that patients with psoriasis had an increased risk of hypertension compared to controls. Finally, Tasliyurt *et al*.[12] found increased evidences favoring the increased prevalence of DM, hypertension, and obesity in psoriasis. Psoriasis is increasingly recognized as a multisystem inflammatory condition associated with a range of co-morbid diseases including obesity, metabolic syndrome, cardiovascular disease, psoriatic arthritis, autoimmune diseases, psychiatric illness, liver disease, and sexual dysfunction.[5] The inflammatory mediators of psoriasis also antagonize insulin signaling, alter adipokine expression, and mediate insulin resistance and obesity.[13]

In the present study, psoriasis severity (PASI score) was significantly higher in females, patients more than 40 years, hypertensive patients, and diabetic patients. Neimann *et al*.[14] found that patients with higher psoriasis severity had higher rates of obesity and DM. Moreover, El-Shahat *et al*.[8] concluded that obesity is directly correlated with psoriasis severity. In addition, Choi *et al*.[5] have shown that DM and hypertension were significantly more prevalent in patients with higher psoriasis severity, and obesity was directly correlated with disease severity. Madanagobalane and Anandan[15] found that hypertension and obesity were more prevalent in those patients with higher disease severity, but there was no correlation between severity of psoriasis and DM. However, Gisondi *et al*.[16] have found that psoriasis was associated with occurrence of DM, hypertension, and obesity independently of its severity. Furthermore, Gupta[17] found that no age or gender differences in the severity of psoriasis were observed.

In our study, single-nucleotide polymorphisms (SNP) of FOXP3 in the peripheral blood of psoriatic patients showed that intron-1 rs3761548 was correlated with a significant susceptibility to

Table 3: FOXP3 genotypes and alleles distribution in patients and controls

Genotype	Patients *n*=80 *n* (%)	Controls *n*=80 *n* (%)	Test of significance	OR (95% CI)
AA (r)	4 (5.0)	11 (13.8)	χ^2=3.6 *P*=0.058	1
AC	25 (31.2)	32 (40.0)	χ^2=1.33 *P*=0.24	2.15 (0.61–7.56)
CC	51 (63.8)	37 (46.2)	χ^2=4.95 *P*=0.026*	3.79 (1.12–12.84)
HWE	χ^2=10.56 *P*=0.01*	χ^2=0.17 *P*=0.6		
Alleles	Patients *n*=160 *n* (%)	Controls *n*=160 *n* (%)		
A (r)	33 (20.6)	54 (33.8)	χ^2=6.96 *P*=0.008*	1
C	127 (79.4)	106 (66.2)		0.132 (0.08–0.219)

χ^2=Chi-square test, **P* value significant <0.05, *r*=reference group, HWE=Hardy- Weinberg equilibrium, A=Adenine, C=Cytosine, OR=Odds ratio

Table 4: Binary logistic regression in prediction of patients

	B	*P*	OR	95.0% CI for OR	
				Lower	Upper
AA (r)			1		
AC	0.765	0.234	2.148	0.610	7.561
CC	1.333	0.032*	3.791	1.119	12.841
Constant	–1.012				

Model χ^2=6.5, *P*=0.039*, percent predicted=58.8%, OR=Odds ratio, A=Adenine, C=Cytosine

psoriasis. There was a significant increase in CC genotype and C allele in patients compared to controls, whereas there were non-significant differences in AA and AC genotypes. In addition, we found that CC genotype is a significant predictor of PV, but, not AC and AA genotypes. Gao *et al*.[4] found five SNPs in the promoter region of FOXP3: −3279C/A (rs3761548), −924A/G (rs2232365), 3499A/G (rs5902434), −1383C/T (rs2232364), and − 2383C/T (rs3761549); the first three types were seen in psoriasis. They found that the risk of psoriasis was markedly increased with the FOXP3 -3279 AC genotype and the combined AC + AA genotype, compared with the -3279 CC genotype.

Shen *et al*.[18] showed that intron-1 rs3761548 was correlated with a significant susceptibility to psoriasis. However, in their study, they showed significant association between psoriasis and genotype AA. Song *et al*.[19] found significant association between PV and FOXP3 polymorphisms (SNPs —rs2232365 A, rs3761547 A, and rs3761549 C) and no correlation between rs3761548 and the onset of PV.

Finally, the present study showed non-significant associations between genotype distributions and each of age, sex, family history of psoriasis, PASI score, hair affection, nail affection, hypertension, DM, and BMI. This indicates that although FOXP3 SNP rs3761548 C may be a risk factor for PV, it is not a risk factor for disease severity or for development of PV comorbidities. However, Gao *et al*.[4] reported that the FOXP3 rs3761548C/A -3279 AC+AA genotype was more obviously associated in males and severe psoriasis patients (PASI score >20). In addition, Song *et al*.,[19] reported that SNPs (—rs2232365 A, rs3761547 A, and rs3761549 C) associated with increased risk of PV in female patients, who were less than 40 years old (early onset psoriasis), had family history of the disease and did not have disease complications.

The differences between the current study and other studies in other countries may be because of the differences in race, genetic background, sample size, gender variability, diet, population age, level of physical activity, levels of over- and undernutrition, body habits, working on other SNPs of FOXP3 gene, and different geographic environments and climates.

Conclusion

FOXP3 gene polymorphism may play a role in psoriasis susceptibility and pathogenesis in Egyptian patients as it leads to changes in FOXP3 protein function and quantity that subsequently affect Tregs count and/or functions. More studies including larger number of Egyptian patients should be done to prove or deny the results of the present study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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