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ORIGINAL CONTRIBUTION

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Angiotensin-converting enzyme gene insertion/deletion polymorphism and family history in severe acne vulgaris

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Summary

Background: Acne vulgaris is an inflammatory disorder with a profound heterogenous aetio-pathophysiology. ACE gene I/D polymorphism affects angiotensin-converting enzyme activities that play a role in inflammation. However, there are no molecular genetic studies investigating the contribution of ACE gene insertion/deletion polymorphism in the genetic background of acne vulgaris.

Aims: The aim of this work was to reveal the relation between the ACE gene I/D polymorphism and acne vulgaris development among a sample of patients.

Patients and Methods: This study included 100 acne vulgaris patients in addition to 120 matched control subjects. The ACE gene I/D polymorphism was analyzed using polymerase chain reaction (PCR).

Results: The distribution of DD, ID genotypes, and D allele showed higher frequency in AV patients than in controls ($P < 0.001$ for all). Moreover, positive family history and ACEI/D gene polymorphism (DD + ID genotypes) were considered as independent predictors for severe acne grades ($P \leq 0.001$ and 0.046, respectively) in multivariate analysis.

Conclusions: The current study results suggest that the D allele of the ACE I/D gene polymorphism might confer risk to AV among the studied patients. Moreover, ACE I/D gene polymorphism and positive family history were considered as independent predictors of severe AV.

KEYWORDS

acne vulgaris, angiotensin-converting enzyme, gene polymorphism

1 | INTRODUCTION

Acne vulgaris (AV) is a pilosebaceous unit disease associated with sebum overproduction, hyperkeratinization of sebaceous follicles, P. acnes colonization, and inflammation.[1] It is mediated by polygenic inheritance or attributed to the interaction between various genes with the environmental factors.[2,3] Although different factors are implicated in the pathophysiology of acne, it is known that oxidative stress is one of the main factors.[4]

Angiotensin-converting enzyme (ACE) gene located on chromosome 17q23 contains an insertion (I) and deletion (D) polymorphism within intron 16. Of the three possible genotypes (DD and II homozygotes and ID heterozygotes), DD genotype carriers have higher serum levels of ACE, than those with the II genotype.[5,6] ACE converts angiotensin I to angiotensin II that could act as a proinflammatory modulator.[7] Moreover, it is a potent activator of the enzyme

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nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) which augments reactive oxygen species (ROS) production, resulting in an oxidative stress state.[8]

The association between ACE gene I/D polymorphism and different skin disorders had been previously investigated[9-12] but it was not investigated in AV. So, we tried to reveal the hypothesized link between ACE gene I/D polymorphism, and AV risk among a sample of patients.

2 | MATERIALS AND METHODS

2.1 | Study population

This case-control study included 100 AV patients and 120 age- and sex-matched control subjects. The diagnosis of AV was based on the patient's history and the typical clinical features. Patients were graded according to Global Acne Grading System (GAGS)[13] into three groups as follows: mild, moderate, and severe.

Patients with other inflammatory diseases as inflammatory bowel disease, chronic active hepatitis, chronic peptic ulcer, or chronic periodontitis; patients suffering from autoimmune diseases as rheumatoid arthritis, systemic lupus erythematosus, thyroid disease, or psoriasis; patients with hypertension or diabetes mellitus; and female patients with menstrual irregularities or history of polycystic ovaries syndrome were excluded from the current study.

Full history taking, general and skin clinical examination, was performed in all patients. Control subjects were recruited during a routine health examination. Peripheral blood samples were collected under full sterile conditions.

This work was approved by the ethical committee on research including human subjects of Benha Faculty of Medicine, and all participants gave informed consents prior to enrollment in the study.

2.2 | DNA extraction and genotyping

Three milliliters venous blood samples was collected from all subjects in EDTA containing tubes. Genomic DNA was extracted by Gene JET® Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, Germany), according to the manufacturer's instructions. PCR was carried out using the I-Star™Taq DNA polymerase enzyme (iNtRON Biotechnology, Korea) and a primer pair: forward primer 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and reverse primer 5′-GATGTGGCCATCAATTCGTCAGAT-3′ (Metabion International AG, Germany) on Veriti thermal cycler (Applied Biosystems). The following PCR conditions were carried out: an initial DNA denaturation at 94°C for two minutes then by 35 cycles of denaturation at 94°C for 20 seconds, annealing at 58°C for 30 seconds and extension at 72°C for 1 minute in addition to final extension at 72°C for 3 minutes. The resultant PCR products (490 bp for insertion and 190 bp for deletion) were separated by 2% agarose gel electrophoresis stained with ethidium bromide to be viewed under ultraviolet illumination (Figure 1).

2.3 | Statistical analysis

The collected data were statistically analyzed using SPSS version 16 software (SPSS Inc, Chicago, IL). Categorical data were presented as number and percentages. Chi-square test (χ^2) or Fisher's exact test (FET) was used to analyze them. Quantitative data were tested for normality using Kolmogorov-Smirnov test, assuming normality at $P > 0.05$.

Normally distributed data were presented as mean ± SD using Student's "t" test or ANOVA test to analyze two or more independent groups, respectively. While nonparametric variables were presented as median and range and analyzed by Kruskal-Wallis test (KW) for difference among three independent means. Univariate and multivariate logistic regression analysis was used to detect the significant predictors of severe AV. The accepted level of significance in this work was stated at 0.05 ($P < 0.05$ was considered significant). Genotype distributions in the studied groups were in Hardy-Weinberg equilibrium for gene polymorphisms (data not shown). Hardy-Weinberg equilibrium was estimated according to OEGE—Online Encyclopedia for Genetic Epidemiology studies.[14]

3 | RESULTS

Studied participants were matched regarding age (19.4 ± 3.7 and 20.1 ± 4.8 years, respectively, $P = 0.231$) and sex (62 [62%] females, 38 [38%] males in patients group, vs 68 [56.7%] female and 52 [43.3%] male in control group, $P = 0.423$; Table 1).

Disease onset was gradual in 74% and the course was progressive in 62% of our studied cases. The reported risk factors among studied patients included sun exposure (68%), family history (60%),

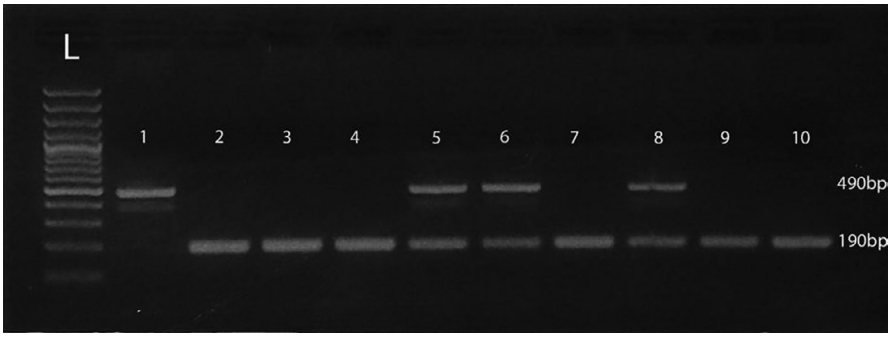

FIGURE 1 A 2% agarose gel electrophoresis shows ACE insertion/deletion polymorphism. Lane 1 shows insertion/insertion genotype. Lanes 2, 3, 4, 7, 9, 10 show deletion/deletion genotype. Lanes 5, 6, 8 show insertion/deletion genotype. L is the DNA ladder

high-glycemic diet (66%), psychological stress (52%), and smoking (7%). The face was affected in all (100%) of our patients, and post-acne scars were present in 38%. Regarding the severity of the disease, the disease was mild in 46%, moderate in 32%, and severe in 22% of studied patients.

The genotypes distribution concerning the ACE I/D polymorphism among control subjects was in Hardy-Weinberg equilibrium as expected (*P* value >0.05).Taking II genotype and I allele as a reference gene, the frequencies of DD and (DD + ID) genotypes and the D allele were higher in patients group (60%, 91%, and 75.5%, respectively) than in control group (25%, 67.5%, and 46.3%) with a statistically significant differences ($P \leq 0.001$ each).Individuals with DD genotype were about 3.7-fold more likely to have acne than patients with II genotype. Also, those with ID genotype were 1.7-fold to have acne than II genotypes. Individuals with D allele were 3.5-fold to have acne than those with I allele (% CI = 2.3-5.3; Table 2).

The relation between genotypes frequency and different clinical parameters is shown in Table 3. No significant relation was found neither between DD genotypes frequency nor ACE gene I/D total polymorphism and most of the clinical parameters among studied patients. However, the DD genotype and ACE I/D gene polymorphism (DD + ID) showed high distribution frequency among patients with severe acne grades ($P = 0.001$ and 0.023, respectively) and positive FH ($P = 0.031$ and 0.041, respectively) when compared to II genotypes frequency.

TABLE 1 Comparison between patients and control groups regarding demographic data

		Control N = 120		AV patients N = 100		*P*
Age (y)	Mean ± SD	20.1	4.7	19.4	3.7	0.231*
	min-max	13	32	14	32	
Males	N, %	52	43.3	38	38	0.423**
Females	N, %	68	56.7	62	62	

*t test.
**Chi-square.

Logistic regression analysis was conducted for prediction of severe acne grades among studied patients using age, gender, smoking, family history, high-glycemic diet, psychological stress, sun exposure, duration of acne, and ACEI/D gene polymorphism (DD + ID genotypes) as risk factors. Positive FH, diet, and ACEI/D gene polymorphism were associated with severe acne grades in univariate analysis ($P = 0.001$, 0.009, and 0.001, respectively). Taking these significant variables into multivariable analysis revealed that positive family history and ACEI/D gene polymorphism were considered as independent predictors of severe acne grades ($P \leq 0.001$ and 0.046, respectively; Table 4).

4 | DISCUSSION

A complex interaction of environmental in addition to genetic factors is involved in AV etiopathogenesis.[15] Previously, genetic investigations were based on testing hypotheses of the possible implication of specific mutations in candidate genes. However, now there are widely available procedures for assessment of the genes and loci due to the advance in genotyping methods allowing genome-wide association studies.[16]

Regarding the relation between ACE gene I/D polymorphism and AV susceptibility, our study results showed that the distribution frequencies of DD genotype and ACE I/D polymorphism (DD + ID) were significantly higher among studied patients than control subjects ($P < 0.001$ and <0.001, respectively). Also in our study, the I allele showed significantly higher frequency in the control group than D allele ($P < 0.001$) indicating that the DD genotype and D allele are associated with AV risk and suggest a protective role of the II genotype. This could be explained as DD genotype carriers have high serum levels of ACE, this favor more production of angiotensin II that augments and perpetuates inflammation and immune response.[7] Angiotensin II increases both the generation of ROS and inflammatory cytokines production such as IL-6[17] and plays an essential role in circulatory homeostasis and inflammation.[18] ACE has been identified in human skin cells like fibroblasts, endothelial cells,

TABLE 2 Comparison between patients and control groups regarding ACE I/D genotypes and alleles frequency

	Control N = 120		AV patients N = 100					
	N	%	N	%	*P*	OR	95% CI	
II	39	33	9	9		1	(reference)	
ID	51	43	31	31	0.022	1.780	1.086	2.918
DD	30	25	60	60	<0.001	3.735	2.289	6.097
ACE gene polymorphism (ID + DD)	81	67.5	91	91	<0.001	2.612	1.664	4.101
I	129	53.8	49	24.5		1	(reference)	
D	111	46.3	151	75.5	<0.001	3.581	2.376	5.397
HW	0.112		0.105					

Abbreviations: CI, confidence interval; HW, Hardy-Weinberg equilibrium; OR, odds ratio.

TABLE 3 Comparison of genotypes frequency and ACE I/D gene polymorphism regarding studied variables and risk factors in AV patients

Variable		II N = 9		ID N = 31		DD N = 60		Total polymorphism (ID + DD) N = 91		*P*1	*P*2
Age	Mean ± SD	20.3 ± 5.2		20.5 ± 4.9		18.7 ± 2.5		19.3 ± 3.5		0.067*****	0.431*
Sex											
Males	N, %	3	33.3	12	38.7	23	38.3	35	38.5	0.955**	0.762**
Females	N, %	6	66.7	19	61.3	37	61.7	56	61.5		
Duration	Median, range	2	0.3-15	2	0.4-16	3	0.2-14	3	0.2-16	0.711****	0.516***
Presence of acne Scars	N, %	2	22.2	14	45.2	22	36.7	36	39.6	0.307**	0.476**
Grading											
Mild	N, %	8	88.9	20	64.5	18	30	38	41.8	0.001**	0.023**
Moderate	N, %	1	11.1	6	19.4	25	41.7	31	34.1		
Severe	N, %	0	0	5	16.1	17	28.3	22	24.2		
Smoking	N, %	2	22.2	6	19.4	6	10	12	13.2	0.292**	0.609**
Family History	N, %	3	33.3	15	48.4	42	70	57	62.6	0.031**	0.041**
Diet	N, %	6	66.7	22	71.0	38	63.3	60	65.9	0.811**	0.965**
Psychological Stress	N, %	5	55.6	18	58.1	29	48.3	47	51.6	0.691**	0.823**
Sun exposure	N, %	5	55.6	18	58.1	35	58.3	53	58.2	0.988**	0.876**

Bold indicates significant *P* values.
*P*1, comparison among II, ID, and DD genotypes; *P*2, comparison between total polymorphism (ID + DD genotypes) vs II genotype.
**t* test.
***Fisher's exact test.
****Mann-Whitney.
*****Kruskal-Wallis.
******ANOVA.

TABLE 4 Logistic regression analysis for prediction of acne severity within all studied patients

	Univariable				Multivariable			
	P	OR	95% CI		*P*	OR	95% CI	
Age[a]	0.214	0.961	0.904	1.023				
Sex	0.421	0.828	0.524	1.311				
Smoking	0.240	1.477	0.770	2.831				
FH	<0.001	3.582	2.155	5.954	<0.001	3.139	1.831	5.380
Diet	0.009	1.913	1.176	3.114	0.268	1.357	0.791	2.330
Psychological stress	0.286	0.784	0.501	1.227				
Sun exposure	0.167	0.728	0.464	1.142				
Duration[a]	0.294	1.035	0.970	1.105				
ACE gene polymorphism (ID + DD genotypes)	<0.001	2.612	1.664	4.101	0.046	3.483	1.024	11.845

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]Cutoff values of age and duration were determined using ROC curve.

and keratinocytes.[19-21] Moreover, it inactivates bradykinin which promotes vasodilation by stimulated synthesis of nitric oxide (NO) that enhances vascular permeability and proinflammatory cytokine production[22] and has been suggested to play a role in the pathogenesis of AV.[5]

This suggests that the DD genotype and the D allele of the ACE I/D gene are associated with increased susceptibility for acne development and was as the same as the condition in various inflammatory disorders where this polymorphism was investigated, including Behcet's disease, vitiligo, and psoriasis.[10-13]

Our results revealed no significant relation between DD genotype and ACE I/D gene polymorphism (DD + ID) vs II genotype and most of the studied clinical parameters and risk factors among studied patients. However, the DD genotype and ACE I/D gene polymorphism (DD + ID) showed higher distribution frequency among patients with severe acne lesions ($P = 0.001$ and 0.023, respectively) and among those with positive family history ($P = 0.031$ and 0.041, respectively) when compared to II genotype carriers.

Although, our results suggest that ACE I/D gene polymorphism might contribute to the genetic susceptibility of AV and could play an integral role in the disease pathogenesis. It was demonstrated that up-regulation of ACE participates in adverse fibrous remodeling. This has been considered a fact in the cardiovascular field and may be implicated in cutaneous scar formation.[22] However, studying the association of ACE I/D gene polymorphism with postacne scarring in our work did not reveal any significant association.

4.1 | Limitations

To our knowledge, this is the first study to investigate the association between the ACE gene I/D polymorphism and AV risk. However, some limitations should be taken into consideration; first, all studied patients were Egyptians and this does not permit extrapolation of our results to other ethnic groups. Second, a small number of patients were included in our study due to the high cost of genotypes analytical molecular studies. Third, the current finding might involve gene-environment interactions which were not explored in our study.

5 | CONCLUSIONS

Our study results suggest that the D allele of ACE I/D gene might confer susceptibility to AV. Moreover, ACE I/D gene polymorphism and positive family history were considered as independent predictors of more severe acne grades. However, due to the small sample size, future studies including larger samples and being multicentric are needed to validate the role of this association among different populations. Such studies might lead to a more comprehensive understanding of the hypothesized association between the ACE I/D gene polymorphism and AV risk.

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