

Association between CYP19A<G rs700518 Polymorphism with Acne Vulgaris and its Severity: Influence on Sex Hormones Level

Ali Ebrahimi¹, Zohreh Rahimi^{2,3*}, Zomorod Ghadami^{1,2}, Ebrahim Shakiba², Ziba Rahimi¹, Mona Akbari¹,
Mehrangiz Shafiei², Fariborz Bahrehmand¹, Asad Vaisi-Raygani⁴, Rozita Naseri⁵

1. Department of Dermatology, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran.

2. Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.

3. Department of Clinical Biochemistry, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran.

4. Fertility and Infertility Research Center, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran.

5. Department of Internal Medicine, Medical School, Kermanshah University of Medical Sciences, Kermanshah, Iran.

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Acne vulgaris (AV) is a common skin disease that causes physical and psychological problems for the affected individual. In addition to systemic changes in hormone levels, overproduction of local steroids, especially androgens are associated with AV. Cytochrome (CYP) 19 is involved in the synthesis of estrogens. The aim of the present study was to investigate the influence of CYP19A <G rs700518 variants on sex hormones level and the risk of AV. In this case-control study, 181 patients with AV and 144 healthy individuals from Western Iran were investigated. The CYP19 variants were detected by PCR-RFLP method. The frequency of GG genotype in all patients (27.1%) was significantly higher than the controls (10.4%, $P < 0.001$), which increased the relative risk of AV by 1.96 times. Also, the presence of this genotype was associated with 1.5-fold ($P = 0.005$), 1.89-fold ($P = 0.011$) and 3.33-fold ($P = 0.019$) increased risk of mild, moderate and severe AV, respectively. A significantly higher serum level of estradiol was observed in women in the presence of AA genotype (107.3 ± 72.1 pg/ml) in comparison with GG genotype (73.2 ± 58.8 pg/ml) ($P = 0.009$). Our study demonstrated that the GG genotype of CYP19 rs700518 polymorphism increased the risk of AV and the severity of the disease, and also was associated with lower levels of estradiol in females. Significantly decreased serum level of sex hormone-binding globulin in women with AV in comparison with healthy individuals could be related to increasing level of androgen that was observed in women with AV.

Key words: Acne vulgaris, CYP19, estradiol, SHBG, androgens

Acne vulgaris (AV) is a chronic inflammatory disease of pilosebaceous unit appearing on

hair follicles and every areas of skin with the most oil (sebaceous) glands. Acne is common in

*Corresponding author: Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.
Email: zrahimi@kums.ac.ir

adolescents but it can be present in adults and children. Some of typical features of acne include seborrhea, papules, inflammation and abnormal follicular keratinization. In addition, acne may cause various degrees of scarring that can be disfiguring lifelong, so it affords negative psychological effects in patient individuals. There are various factors like stress, hormonal or genetic factors, drugs, cosmetics, irritation and potential dietary factors, which cause inflammation and formation various types of acne lesions (1, 2).

Cytochromes (CYPs) P450 comprise a superfamily of microsomal hemoproteins that catalyze the phase I biotransformation of many endogenous substrates and xenobiotics. Various form of CYP enzymes are found in all eukaryotic organisms, animals, plants, and fungi (3). In human, CYPs are present in the endoplasmic reticulum (microsomes) (4). A wide range of metabolic capacity is provided due to the genetic variations of cytochrome isoenzymes (5). This family includes 57 genes that encode for enzymes that can have effect on drugs, arachidonic acid and eicosanoids, foreign chemicals, cholesterol, and steroids metabolism (6). Six CYP enzymes take part in steroidogenesis. When sexual differentiation occurs in early embryogenesis, the transcription factor steroid-factor-1 plays an important role in upregulation of CYP genes involved in steroid hormone synthesis. These genes include members of the CYP11, CYP17, CYP19 and CYP21 families (6). The CYP19A1 is located within the endoplasmic reticulum and synthesizes estrogen by aromatization of A ring of the androgenic steroid substrates. Mutations in this gene can increase or decrease aromatase activity, sex hormone levels (estrogen levels) and bone mineral density and fractures (7).

The CYP19 polymorphism (rs700518) is an anonymous variation in exon 3 (Val 80 Val), which can affect the post-transcriptional processing, and results in changes in gene expression, aromatase

levels, and thereby on estrogen production (8). According to literature there is no available study to investigate the role of CYP19 polymorphism (rs700518) in the pathogenesis of AV and its effect on the sex hormones level.

The present study aimed to find the association between the CYP19 variants with sex hormones level and with the risk of AV in a population from Western Iran.

Patients and methods

Patients

In this case-control study, 181 individuals with AV including 154 females and 27 males and 144 healthy individuals (113 females and 41 males) without systemic and dermatologic disorders were investigated. All studied individuals were examined by dermatologist. Among patients with AV there were 82 with mild AV, 48 with moderate AV and 51 patients with severe AV. The clinical grade of AV was determined as mild with the presence of comedones without significant inflammation and a few or no papules; moderate with the presence of comedones and significant inflammatory papules and pustules; and severe with the presence of comedones, papules and pustules and inflammatory nodules (9). All studied individuals were from Kermanshah province in Western Iran. The basic information of all subjects including age, sex, age of the disease onset, the severity of AV symptoms, menstrual pattern and BMI were collected.

Informed written consent was obtained from each individual before participation in the study. The study was approved by the Ethics Committee of Kermanshah University of Medical Sciences and was in accordance with the principles of the Declaration of Helsinki II.

Biochemical analyzes

The serum levels of triglycerides (TG), cholesterol, fasting blood sugar (FBS), low and high density lipoprotein-cholesterol (LDL-C and HDL-C, respectively) were measured using the Bionic Diagnostic Kits (Iran) by using the Mindrey

BS-480 chemistry analyzer. Serum estradiol in the mid-follicular phase of the menstrual cycle, dehydroepiandrosterone (DHEA) and sex hormone binding globulin (SHBG) levels were measured by the chemiluminescent method using the Abbott Architect i1000 (Abbott Laboratory, USA).

Genotyping

Standard method of phenol-chloroform was used to extract DNA from whole blood of each individual (2, 10). The CYP19 (rs700518) variants were determined by polymerase-chain reaction (PCR) restriction fragment length polymorphism (RFLP) using the forward primer 5'-AGTAACA-CAGAACAGTTGCA-3' and the reverse primer 5'-TCCAGACTCGCATGAATTCTCCGTA-3'. The final PCR product in the presence of a DNA ladder (50 bp) was electrophoresed on 2% agarose gel. The CYP19 AA genotype was identified by a single band of 188-bp, a band of 164-bp indicated the GG

genotype and the double bands of 164-bp and 188-bp demonstrated the AG genotype (8).

Statistical analysis

The allelic frequencies were calculated by the chromosome counting method. The significance of differences in genotype and allele frequencies of CYP19 polymorphism between patients and controls were calculated using the χ^2 test. The odds ratios (OR) was calculated as the estimates of relative risk for disease, and 95% confidence intervals (CI) were obtained by SPSS logistic regression software. A two-tailed student's t test was used to compare quantitative data. The SPSS (SPSS Inc., Chicago, IL, USA) statistical software package version 22.0 was used for statistical analysis.

Results

Demographic and biochemical characteristics

Table 1. Characteristics of patients and controls.

Variables	Female Patients n=154	Female Controls n=113	Male Patients n=27	Male Controls n=41
Age (years)	22.3±4.6 P=0.29	22.7±4.1	21.3±4.9 P=0.3	22.3±4.3
BMI (Kg/m ²)	23.4±6.4 P=0.6	22.7±3.9	23.1±3.3 P=0.11	22.2± 2.6
SHBG (nmol/l)	48.6±24.1 P=0.002	56.4±32.4	31.9±15.2 P=0.026	46.6± 30.6
Estradiol (pg/ml)	76.5±68 P=0.019	98.9±81.6	35.8±22.1 P=0.11	48.5± 41.2
DHEA (µg/dl)	645.4±153.2 P=0.047	277.1±4630	332.4± 166.4 P=0.09	1660.6± 8572.8
FBS (mg/dl)	81.6±10.8 P=0.15	78.4±18.3	88.4± 9 P=0.87	84.30± 8.6
Cholesterol (mg/dl)	133.3±31.5 P=0.72	127.2±32.2	125.8± 25 P=0.06	141.1± 34.3
TG (mg/dl)	85±52.9 P=0.41	86.9±44	94.3± 57.7 P=0.5	101.7± 44.7
HDL-C (mg/dl)	49.3±12.6 P=0.044	40.6±9.9	44.8± 9.3 P=0.38	41.7± 11.9
LDL-C (mg/dl)	76.9±26.3 P=0.31	71.6±24.3	71.4± 22.3 P=0.25	78.6± 28.3

BMI: body mass index; DHEA: dehydroepiandrosterone; FBS: fasting blood sugar; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; SHBG: sex hormone binding globulin; TG: triglycerides.

Table 2. Comparison of the frequency of CYP19 rs700518 genotypes and alleles between patients with acne vulgaris and controls.

	Patients n=181 (%)	Mild Acne n=79 (%)	Moderate Acne n=49 (%)	Severe Acne n=53 (%)	Controls n=144 (%)
CYP19 Genotypes					
AA	40 (22.1)	18 (22.8)	11(22.4)	11 (20.8)	46 (31.9)
AG	92 (50.8)	39 (49.4)	24(49)	29 (54.7)	83 (57.6)
	* $\chi^2=0.85$, p=0.35	22 (27.8)	14 (28.6)	13 (24.5)	15 (10.4)
GG	49 (27.1)	* $\chi^2=8.2$, p=0.004	* $\chi^2 =6.7$, p=0.01	* $\chi^2=5.8$, p=0.016	
	* $\chi^2 =13.7$, p<0.001	*OR=1.5 (95% CI	*OR=1.89 (95% CI	*OR=3.33 (95% CI	
	*OR=1.96(95% CI	1.7-2, p=0.005)	1.15-3.08, p=0.011)	1.22-8.9, p=0.019)	
	1.36-5.42, p<0.001)				
CYP19 Alleles					
A	172 (47.5)	75 (47.5)	46 (46.9)	51 (48.1)	175 (60.8)
G	190 (52.5)	83 (52.5)	52 (53.1)	55 (51.9)	113 (39.2)
	$\chi^2=11.3$, p= 0.001, OR= 1.71 (95%CI	$\chi^2=6.06$,p=0.014 OR= 1.64	$\chi^2=4.77$, p=0.029 OR= 1.67 (%95CI	$\chi^2=4.2$, p= 0.041 OR= 1.6 (%95CI	
	1.25-2.34, p=0.001)	(%95CI 1.1-2.43, p=0.014)	1.05-2.66, p=0.03)	1.02–2.51, p=0.042)	

Overall, $\chi^2=14.9$, P = 0.001 comparing three genotypes between patients and controls

*Compared to AA genotype between patients and controls.

of women and men (patient and control groups) are represented in Table 1. In women with AV, the levels of SHBG (48.6±24.1 nmol/l, P = 0.002) and estradiol (76.5±68 pg/ml, P = 0.019) were significantly lower than healthy women (56.4±32.40 nmol/l, 98.9±81.6 pg/ml, respectively). Also, significantly higher levels of DHEA (645.4±153.2 µg/dl, = 0.047) and HDL-C (49.30±12.6 mg/dl, P = 0.044) were observed in women with AV in comparison with controls (277.1±4630.2 µg/dl and 40.6±9.9 mg/dl, respectively) (Table 1).

On the other hand, among men, only serum concentration of SHBG was significantly higher in controls (46.6± 30.60 nmol/l, P = 0.026) than patients (31.9± 15.2 nmol/l) (Table 1).

Comparing different types of AV in women demonstrated that the level of SHBG tended to decrease with increasing AV severity (mild AV=50.8±23.1, moderate AV=49.4±27.5, and severe AV=44.7±22.1 nmol/l). Also, among women there was the same trend toward decreasing estradiol level

in mild to severe AV (mild AV=86.5±82.7, moderate AV=78.6±65, and severe AV=61.2±44.3 pg/ml).

The frequency of CYP19 rs700518 genotypes and alleles in all patients, patients with mild-, moderate- and severe- AV and controls are presented in Table 2. As shown in this table, the frequency of GG genotype in all patients (27.1%) was significantly higher than controls (10.4%, P<0.001), which increased the relative risk of AV by 1.96 times. Also, the presence of this genotype was associated with 1.5-fold (P = 0.005), 1.89-fold (P = 0.011) and 3.33-fold (P = 0.019) increased risk of mild-, moderate- and severe-AV. Further, the frequency of G allele in patients (52.5%) was significantly higher than controls (39.2%, P<0.001). This allele increased the relative risk for AV by 1.71 times (P = 0.001).

Analysis of all studied women indicated a significantly higher serum level of estradiol in the presence of AA genotype (107.3±72.1 pg/ml) compared to GG genotype (73.2±58.8 pg/ml) (P =0.009).

Discussion

The present study revealed a lower serum level of SHBG in men and women affected with AV in comparison with healthy individuals. Also, in women with AV the levels of estradiol and DHEA were significantly lower and higher, respectively than the healthy women. Comparing different types of AV in women indicated that the levels of SHBG and estradiol decreased with increasing the AV severity.

The real cause of AV is unknown. It has been suggested that the onset of molecular reactions in the skin is affected by genetic and hormonal factors. These factors probably provide suitable environment for growth and proliferation of acne's follicle or affect on inflammatory responses. (11). The sebaceous glands (or sebum) contain a large number of receptors for androgens, estrogens, thyroid stimulating hormone, prostaglandins, and so on (12). Sebum is an important site for active androgens formation. Increasing level of androgens, especially during puberty, can increase the size of the sebaceous glands and produce abnormal and excessive sebum inside the gland, as well as increasing the horny skin. This extra sebum can lead to the closure of the follicle and the formation of acne. The role of estrogens including estradiol in the development of AV is not very clear. Estrogens may directly oppose the effects of androgens in the sebaceous glands, inhibit androgen production by gonadal tissue through a negative feedback regulation to release the pituitary gonadotropin, or adjust genes that negatively affect on sebaceous gland growth or suppressing lipid fatty production (13).

The level of SHBG in blood was shown to be dependent on gender, age, testosterone and estrogen levels, and some diseases such as liver disease, hypothyroidism, and obesity (14).

It has been reported that increasing DHEA level is associated with the presence of acne (15). Moreover, higher levels of total testosterone, free

testosterone and progesterone, cholesterol and LDL-C and lower levels of SHBG, estradiol and HDL-C are significantly associated with severe AV. So, changes in lipid profile and hormone levels can be considered in treatment of female patients with moderate to severe AV (16).

The lower serum levels of SHBG and estradiol and the higher level of DHEA in females with AV compared to the control group and their association with the severity of AV in the present study indicates that decreased level of estradiol and increased level of androgens are associated with decreasing SHBG level and also are related to the risk of AV and its severity. It also appears that abnormal lipid profile in women could be associated with AV.

The CYP19 gene encodes the aromatase enzyme which catalyzes the conversion of androgen into estrogen, which is related to endometriosis development (17). Theoretically, any alteration in CYP activity changes the circulating estradiol level (7, 18). The specific tissue expression of CYP19 gene is regulated by specific tissue promoters and by alternative splicing mechanisms (19).

The CYP19 polymorphism (rs700518) is an A/G base change at Valine 80 on exon 3 and might be effective on post-transcriptional regulation, leading to an alteration in gene expression, aromatase levels, and thereby on estrogen production (7). It has been reported that the AA genotype of rs700518 was associated with some estrogen-dependent diseases such as hypertension, breast cancer and endometriosis (8). So far, no study has looked at the association between AV and CYP19 rs700518. However, Chamaie-Nejad et al. (20) investigated an association between CYP19 T>C and AV and reported that the TC genotype increased the risk of overall AV and mild AV by 2.1 and 3.2 times, respectively.

Our study demonstrated that the frequencies of CYP19 GG genotype and G allele in the patients group were significantly higher than the control

group, which increased the risk for AV by 1.94 and 1.71 times, respectively. Also, based on the disease severity, the presence of GG genotype significantly increased the risk of severe form more than the mild form of the disease. Further, in females with GG genotype the estradiol level was significantly lower in comparison with those having AA genotype.

An investigation on 359 Caucasian females detected significant associations for five SNPs of CYP19, including rs700518, with the age at menarche (21). Peter et al. (22) reported a significant association between variations in the aromatase gene (CYP19A1) and estradiol and testosterone levels, and the estradiol to testosterone ratio in men. In addition, a prospective observational study on postmenopausal women with early breast cancer exhibited association of variants in CYP19A1 with decreased triglycerides and variable changes in HDL-C (23). Also, another prospective observational study in patients with positive estrogen receptor breast cancer in Italy showed that patients with AA genotype for CYP19A1 rs700518 were at risk for aromatase inhibitor-associated bone loss and deserved close follow-up during long-term aromatase inhibitors therapy (7).

In summary, our study demonstrated that the GG genotype of CYP19 rs700518 increased the risk of AV and the severity of the disease, and was also associated with lower levels of estradiol in females. Also, there was a significant reduced level of estradiol in women with AV in comparison with controls. Significant decreased serum levels of SHBG in women with AV in comparison with healthy individuals could be related to increasing levels of androgens that were observed in women with AV.

Conflict of interest

The authors declare that they have no conflict of interest.

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