Vascular endothelial growth factor gene polymorphisms in patients with rosacea: A case-control study

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Background: Rosacea is a chronic disease that is characterized by facial skin inflammation and vascular abnormality. Vascular endothelial growth factor (VEGF) is a potent mediator of vascular permeability and inflammation that might play a role in the pathogenesis of rosacea.

Objective: This study aimed to determine the association between VEGF gene polymorphisms and rosacea.

Methods: A case-control study design was used to compare 100 patients with rosacea and 100 age- and gender-matched control subjects in terms of VEGF polymorphisms based on polymerase chain reaction and the serum level of VEGF and VEGF receptors based on enzyme-linked immunosorbent assay.

Results: Heterozygous and homozygous +405C/G polymorphism of the VEGF gene was observed to increase the risk of rosacea 1.7-fold (95% confidence interval 1.2-4.2) and 2.3-fold (95% confidence interval 1.2-4.2), respectively. There was a significant positive correlation between the severity of rosacea and +405C/G polymorphism of the VEGF gene in patients with erythematotelangiectatic rosacea.

Limitations: Serum VEGF and VEGF receptor levels were measured in the limited number of patients.

Conclusion: The present findings indicate that +405C/G polymorphism of the VEGF gene increases the risk of rosacea. (J Am Acad Dermatol 2019;81:348-54.)

Key words: rosacea; vascular endothelial growth factor; VEGF gene polymorphism.

INTRODUCTION

Rosacea is a chronic inflammatory disease characterized by erythema, telangiectasia, papules, and pustules that generally involves the midfacial region. The prevalence of rosacea varies with ethnicity, age, gender, and geography, ranging from 1% to 22%.[1-3] Although the etiology of rosacea remains unclear, genetic factors, immune system, hormones, and environmental factors are thought to play a role in its pathogenesis.[4,5] Flushing, a gradual increase in facial erythema, and alterations in vascular response to physicochemical stimuli in the majority of patients, as well as vascular abnormalities based on videocapillaroscopy or seen on biopsy specimens suggest that vascular changes might also play a role in the pathogenesis of rosacea.[6-8]

In patients with rosacea, numerous mediators, including nitric oxide, cathelicidins, and vascular endothelial growth factor (VEGF), are involved in vasodilatation and angiogenesis.[8] In addition to its physiological importance, VEGF is the most essential positive regulator of pathological angiogenesis and increased vascular permeability in patients with inflammatory diseases and tumors.[9-12] VEGF is also

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expressed in normal epidermis and skin adnexa, which suggests that it might play a role in the pathogenesis of rosacea.[13,14] Although the published literature includes numerous studies on the role of vascular changes and inflammation in the pathogenesis of rosacea, only a few have investigated the role of VEGF.[15-17]

The present study aimed to determine the association between VEGF polymorphisms and rosacea via comparison of +405C/G, −460T/C, −1540C/A, −1512 Ins18, and −1451C/T polymorphisms of the VEGF gene in patients with rosacea and control subjects. These particular polymorphisms were chosen for analysis because they are reported to be functionally relevant in many inflammatory diseases that have a vascular component.[16-22] An additional aim was to compare serum VEGF, VEGF receptor-1 (VEGFR-1)/fms-like tyrosine kinase (FLT-1), and VEGFR-2/kinase insert domain receptor (KDR) levels between patients with rosacea and control subjects.

CAPSULE SUMMARY

- Studies investigating vascular endothelial growth factor in patients with rosacea showed elevated tissue vascular endothelial growth factor expression in rosacea, and our study showed that vascular endothelial growth factor polymorphism (+405C/G) increases the risk of rosacea.
- Vascular endothelial growth factor may be a new target for future rosacea treatments.

METHODS

Participants

The patient group included 100 adults who were 18 to 73 years of age and who were diagnosed with rosacea. The control group consisted of 100 adults that were examined because of skin diseases other than rosacea. Exclusion criteria included inflammatory arthropathy, malignancy, and pregnancy or nursing an infant. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Hacettepe University Ethics Committee (GO 2013/13-108). Written informed consent was provided by each participant.

Patient demographic data, Fitzpatrick skin phototype, rosacea subtype and severity, medical history, and clinical and laboratory findings were recorded. An ophthalmologic examination was performed in all patients with rosacea and ocular involvement was graded on a 4-point Likert-type scale, as follows: 0, no involvement; 1, mild involvement; 2, moderate involvement; and 3, severe involvement.

VEGF genotyping

DNA was isolated from each participant's whole blood using a Qiagen Qiaamp DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany). Polymerase chain reaction was carried out for 5 VEGF gene polymorphisms (+405C/G, −460T/C, −1540C/A, −1512 Ins18, and −1451C/T), using specific primers and conditions.[23] Amplified products were digested with *Bg1*II for VEGF polymorphisms −1540C/A and −1512Ins18 and were digested with *BstU*I, *BsmF*1, and *Bfa*I for VEGF polymorphisms −460T/C, +405C/G, and −1451C/T, respectively (New England BioLabs Inc, Hitchin, United Kingdom). Appropriate DNA fragments corresponding with the VEGF polymorphism alleles were separated via agarose gel electrophoresis and then visualized using ethidium bromide.

Serum VEGF, VEGFR-1/FLT-1, and VEGFR2/KDR levels

Serum VEGF, VEGFR-1/FLT-1, and VEGFR-2/KDR levels were measured via enzyme-linked immunosorbent assay using a Boster kit (Boster Immunoleader; Boster Biological Technology Co, Ltd, Abingdon, United Kingdom).[18]

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows (v 21.0; IBM Corp, Armonk, NY). Numerical variables are presented as mean ± standard deviation (SD) and categorical variables are presented as number and percentage. A paired group comparison of independent groups was performed using the Student *t* test. Categorical variables were compared using the chi-squared, Fisher exact, or Mantel−Haenszel tests, as appropriate. The Mann-Whitney *U* test was used for paired group comparison of numerical variables when the variables were not normally distributed. Correlation analysis was performed using the Spearman correlation test. The additive model of genotype distribution on rosacea (the additional risk of rosacea associated with each alternate allele) was analyzed via multiple logistic regression, whereas the dominant model (disease risk of heterozygote plus homozygote vs. normal) and recessive model (disease risk of homozygote versus heterozygote plus normal) were analyzed via the chi-squared test. Odds ratios with 95% confidence intervals were used to describe the risk of rosacea. Hardy−Weinberg equilibrium was analyzed using the 1-sample chi-squared test, with 1 degree of

Table I. Distribution of VEGF polymorphisms in the rosacea and control groups

	Rosacea group, n = 100	Control group, n = 100	*P* value
+405 C/G polymorphism, n (%)			
Normal	55 (55.0)	71 (71.0)	.017*
Heterozygous	20 (20.0)	15 (15.0)	
Homozygous	25 (25.0)	14 (14.0)	
−460 T/C polymorphism, n (%)			
Normal	72 (72.0)	71 (71.0)	.88*
Heterozygous	26 (26.0)	29 (29.0)	
Homozygous	2 (2.0)	0 (0.0)	
−1540C/A polymorphism, n (%)			
Normal	77 (77.0)	79 (79.0)	.73*
Heterozygous	23 (23.0)	21 (21.0)	
Homozygous	0 (0.0)	0 (0.0)	
−1451C/T polymorphism, n (%)			—
Normal	100 (100.0)	100 (100.0)	
−1512Ins18 polymorphism, n (%)			
Normal	64 (64.0)	72 (72.0)	.23†
Ins	36 (36.0)	28 (28.0)	

VEGF, Vascular endothelial growth factor.
*Mantel−Haenszel test.
†Fisher exact test.

Table II. The Hardy−Weinberg equilibrium for each VEGF gene polymorphism in the rosacea and control groups

	Rosacea group		Control group	
VEGF polymorphism	Chi-squared	*P* value*	Chi-squared	*P* value*
+405C/G	31.4	<.001	30.9	<.001
−460T/C	0.04	.84	2.9	.09
−1540C/A	1.7	.19	1.4	.24
−1451C/T	NA†	NA†	NA†	NA†
−1512Ins18	4.8	.028	2.7	.10

NA, Not applicable; *VEGF*, vascular endothelial growth factor.
*Degree of freedom = 1.
†Polymorphism was not identified in any individual.

freedom. The level of statistical significance was set at $P < .05$.

RESULTS

The study included 100 patients with rosacea and 100 control subjects. The mean age of the patients was 44.6 ± 12.5 years versus 44.6 ± 12.8 years for the control subjects ($P = .98$). In all, 77% of the patients with rosacea were female and 23% were male; gender distribution did not differ significantly between the patient and control groups ($P > .99$). The mean disease duration in the patient group was 9.4 ± 9.1 years. The most common rosacea type was erythematotelangiectatic (ET; 63%), followed by papulopustular (25%) and phymatous rosacea (9%). Among the patients with rosacea, 22% were Fitzpatrick skin phototype 1, 40% were Fitzpatrick skin phototype 2, 36% were Fitzpatrick skin phototype 3, and 2% were Fitzpatrick skin phototype 4. There was a positive family history of rosacea in 44% of the patients. In all, 95% of the patients had ocular involvement, which was mild to moderate in 98.9%; the eyelids and the meibomian gland were the most commonly affected (99.1%) regions, followed by inflammation of the ocular surface (69.7%).

The distribution of VEGF gene polymorphisms in the patient and control groups is summarized in Table I. The prevalence of both homozygous (GG) and heterozygous (CG) +405C/G polymorphisms of the VEGF gene was significantly higher in the patient group than in the control group ($P = .017$). There was not a significant difference in the prevalence of VEGF gene −1540C/A, −1512Ins18, and −460T/C polymorphisms between the patient and control groups. CG +405C/G polymorphism of the VEGF gene increased the risk of rosacea 1.7-fold (95% confidence interval 1.1-3.6), whereas GG +405C/G polymorphism increased the risk 2.3-fold (95% confidence interval 1.0-4.2).

There was a significant correlation between the severity of ET rosacea and VEGF gene +405C/G polymorphism and between the severity of phymatous rosacea and VEGF gene −460T/C polymorphism. The severity of ET and phymatous rosacea increased as the number of polymorphic alleles increased ($r = 0.22$ and $P = .002$, and $r = 0.23$ and

Table III. Serum VEGF, VEGFR-1/FLT-1, and VEGFR-2/KDR levels in the rosacea and control groups

	Rosacea group		Control group		
Serum levels	**n**	**Median (IQR) pg/mL**	**n**	**Median (IQR) pg/mL**	***P* value**
VEGF	20	179 (151-219)	20	227 (150-271)	.176
VEGFR-1/FLT-1	10	213 (171-275)	10	170 (139-196)	.070
VEGFR-2/KDR	10	430 (387-523)	10	361 (285-444)	.112

FLT-1, fms-like tyrosine kinase; *IQR*, interquartile range; *KDR*, kinase insert domain receptor; *VEGF*, vascular endothelial growth factor; *VEGFR*, vascular endothelial growth factor receptor.

Table IV. Correlations between VEGF gene polymorphisms and serum VEGF, VEGFR-1/FLT-1, and VEGFR-2/KDR levels

	VEGF gene polymorphism			
Serum levels (pg/mL)	**+405C/G**	**−460T/C**	**−1540C/A**	**−1512Ins18**
VEGF				
r	−0.060	−0.035	−0.108	−0.076
P value	.713	.831	.509	.643
n	40	40	40	40
VEGFR-1/FLT-1				
r	0.083	0.039	0.416	−0.340
P value	.727	.870	.068	.142
n	20	20	20	20
VEGFR-2/KDR				
r	0.180	−0.091	0.089	0.157
P value	.448	.702	.711	.509
n	20	20	20	20

FLT-1, fms-like tyrosine kinase; *KDR*, kinase insert domain receptor; *VEGF*, vascular endothelial growth factor; *VEGFR*, vascular endothelial growth factor receptor.

P = .012, respectively). There was not a significant correlation between disease severity and +405C/G polymorphism of the VEGF gene in patients with other subtypes of rosacea. The incidence of VEGF polymorphism did not differ significantly between the patients with and without a family history of rosacea. Significant deviation from the Hardy–Weinberg equilibrium for VEGF gene +405C/G polymorphism was not observed in the patient or control groups; however, there was significant deviation for VEGF gene −1512Ins18 polymorphism in the patient group (Table II).

Regarding the serum level of VEGF and VEGFRs (VEGFR-1/FLT-1 and VEGFR-2/KDR), the VEGF and VEGFR-2/KDR levels (Table III) did not differ significantly different between the patient and control groups, whereas the VEGFR-1/FLT-1 level was higher (approaching the level of significance) in the patient group (P = .068). Lastly, a moderate correlation (approaching the level of significance) was noted between the VEGFR-1/FLT-1 level and the −1540C/A polymorphism of the VEGF gene (Table IV).

DISCUSSION

The VEGF gene is highly polymorphic, with >25 documented types of polymorphism.[21] These polymorphisms have been investigated in numerous diseases in which angiogenesis plays a role in the pathogenesis, such as diabetic retinopathy, breast cancer, coronary artery disease, rheumatoid arthritis, and psoriasis.[18,22-25] The present study investigated VEGF gene polymorphisms in patients with rosacea, a chronic skin disease with both inflammatory and vascular components.

Our findings show that the prevalence of +405C/G polymorphism of the VEGF gene was higher in patients with ET, papulopustular, and phymatous rosacea than in the control subjects. The risk of rosacea associated with this polymorphism was 1.7- and 2.3-fold higher in CG and GG patients, respectively, then in control subjects. VEGF +405C/G polymorphism was also significantly associated with disease severity in the patients with the ET type of rosacea. These findings show that VEGF polymorphisms are a risk factor for all types of rosacea, and that the risk is highest for the ET type. Similarly, it was reported earlier that +405C/G polymorphism of the VEGF gene is a risk factor for abnormal coronary microvasculature, rheumatoid arthritis, and Behçet disease.[26-28] In addition, an animal study by Hirakawa et al[29] showed that the VEGF overexpression increased ultraviolet B light–induced erythema, edema, vascular dilatation, and inflammatory cell infiltration. Their findings also partly support the association of VEGF with disease severity, because ultraviolet B light is a well-known aggravating factor for rosacea.[29]

To the best of our knowledge, the present study is the first to examine serum VEGFR-1 and VEGFR-2 levels in rosacea patients. The serum levels of VEGFR-1 and VEGFR-2 in the present study were higher in patients with rosacea than in the control group, but not significantly, although the difference in the VEGFR-1 level approached the level of significance. VEGF binds to VEGFR-1 and VEGFR-2 on hematopoietic and endothelial cells, as well as to soluble VEGFR-1. In hematopoietic cells, VEGFR-1

and VEGFR-2 induce cell migration and survival, but in endothelial cells these 2 receptors function differently. VEGFR-2 facilitates migration, proliferation, survival, and angiogenesis in vascular endothelial cells.[30] VEGF binds to VEGFR-1 with greater affinity than to VEGFR-2, but VEGFR-1 has a lower level of tyrosine kinase activity, and it is hypothesized that VEGFR-1 and its soluble form (sVEGFR-1) are decoy receptors that prevent VEGF from binding to VEGFR-2.[30-36] In their study, Chappell et al[37] described this inhibitory effect as a local guidance system, where sVEGFR-1 expression was increased in growing and branching vessels. The increased sVEGFR-1 was observed on lateral sites of developing vessels. Chappell et al[37] postulated that sVEGFR-1 enhances sprout emergence, the type of morphogenesis during blood vessel formation, by inhibiting VEGF activity on lateral sites and leaving an uninhibited middle path for the vessel to branch. Smith et al[17] demonstrated an increased expression of VEGFR-1 in rosacea skin samples, and VEGFR-1 may contribute to formation of abnormally branching capillaries seen in rosacea. The increased serum VEGFR-1 levels shown in our study may reflect the localized tissue expression of sVEGFR-1. The need to further enlighten the role of VEGFR-1 in pathogenesis of rosacea remains.

A Turkish study reported that the serum VEGF concentration did not differ significantly between patients with rosacea and control subjects but that the VEGF concentration in tears was lower in patients with rosacea.[38] The researchers attributed these findings to the absence of ocular vascularization or the presence of corneal complications in the patients with rosacea. In the present study, the serum VEGF level did not differ significantly between the patient and control groups. VEGF binds to VEGFR-1 and VEGFR-2, inducing both physiological and pathological angiogenesis, as well as inflammation. Elevated VEGF and VEGFR expression in tissue supports the hypothesis that VEGF might play a role in the pathogenesis of rosacea as an angiogenetic and proinflammatory mediator. Lachgar et al[39] observed a dose-dependent decrease in VEGF expression in keratinocyte cell cultures in response to the administration of retinoid acid and suggested that the effects of isotretinoin treatment in rosacea patients might occur via VEGF.[40]

Two earlier studies showed that +405C/G polymorphism of the VEGF gene can play a role in the pathogenesis of a disease by affecting the serum VEGF level. Both Watson et al[41] and Stevens et al[42] reported that VEGF production is positively correlated with the G allele of the +405C/G polymorphism, with the highest VEGF levels observed in individuals with the GG genotype, followed by the GC and CC genotypes. In the present study, a correlation between the G allele of +405C/G polymorphism of the VEGF gene and the VEGF level was not observed, which might have been because of the small patient population and the consequent low study power for analyzing this association along with its confounders, or it might be indicative of the fact that the VEGF gene is highly polymorphic and that other gene polymorphisms may alter VEGF production. Likewise, behavioral factors like smoking and body mass index were also shown to interact with the serum concentration of this cytokine.[39,43]

The literature includes only a few studies on tissue levels of VEGF and VEGFR in patients with rosacea. Among them, 3 studies focused on VEGF expression in skin biopsy specimens obtained from patients with rosacea, and all reported elevated VEGF expression in active rosacea.[16,17,44] In addition, all 3 studies indicated that elevated tissue VEGF expression might contribute to the increase in vascularity observed in rosacea patients. Kajiya et al[44] investigated VEGF expression in reddened skin, an early state of facial erythema that can progress to rosacea. The study showed increased angiogenesis along with increased expression of VEGF in epidermis of reddened skin suggesting the possible role of VEGF in reddened skin. Kajiya et al[44] also suggested that VEGF could be a target for topical treatments of reddened skin and early rosacea.

In the present study, expression of VEGF and VEGFR in biopsy tissue samples was not studied, which is a limitation. Additional larger-scale studies that investigate tissue VEGF and VEGFR levels are needed to better understand the role of VEGF in rosacea. Another limitation of the present study is that biochemical analysis of serum VEGF, VEGFR-1, and VEGFR-2 was performed in only a limited number of patients with rosacea. In addition, the study included only Turkish patients with rosacea, and because VEGF polymorphisms might vary markedly according to ethnic group, the generalizability of the present findings is limited.

In conclusion, +405C/G polymorphism of the VEGF gene increases the risk of rosacea; however, additional research with larger multiethnic patient and control groups that investigates VEGF and VEGF receptor concentrations in biopsy tissue samples is needed to further clarify the relationship between VEGF and rosacea.

REFERENCES

1. Abram K, Silm H, Oona M. Prevalence of rosacea in an Estonian working population using a standard classification. *Acta Derm Venereol*. 2010;90:269-273.

2. Doe PT, Asiedu A, Acheampong JW, et al. Skin diseases in Ghana and the UK. *Int J Dermatol*. 2001;40:323-326.
3. Schaefer I, Rustenbach SJ, Zimmer L, et al. Prevalence of skin diseases in a cohort of 48,665 employees in Germany. *Dermatology*. 2008;217:169-172.
4. Steinhoff M, Buddenkotte J, Aubert J, et al. Clinical, cellular, and molecular aspects in the pathophysiology of rosacea. *J Investig Dermatol Symp Proc*. 2011;15:2-11.
5. Yamasaki K, Gallo RL. The molecular pathology of rosacea. *J Dermatol Sci*. 2009;55:77-81.
6. Rosina P, Zamperetti MR, Giovannini A, et al. Videocapillaroscopic alterations in erythematotelangiectatic rosacea. *J Am Acad Dermatol*. 2006;54:100-104.
7. Aroni K, Tsagroni E, Kavantzas N, et al. A study of the pathogenesis of rosacea: how angiogenesis and mast cells may participate in a complex multifactorial process. *Arch Dermatol Res*. 2008;300:125-131.
8. Del Rosso JQ. Advances in understanding and managing rosacea: part 1: connecting the dots between pathophysiological mechanisms and common clinical features of rosacea with emphasis on vascular changes and facial erythema. *J Clin Aesthet Dermatol*. 2012;5:16-25.
9. Bowden J, Brennan PA, Umar T, et al. Expression of vascular endothelial growth factor in basal cell carcinoma and cutaneous squamous cell carcinoma of the head and neck. *J Cutan Pathol*. 2002;29:585-589.
10. Brown LF, Berse B, Jackman RW, et al. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. *Cancer Res*. 1993;53:4727-4735.
11. Detmar M, Brown LF, Claffey KP, et al. Overexpression of vascular permeability factor/vascular endothelial growth factor and its receptors in psoriasis. *J Exp Med*. 1994;180:1141-1146.
12. Ferrara N, Frantz G, LeCouter J, et al. Differential expression of the angiogenic factor genes vascular endothelial growth factor (VEGF) and endocrine gland-derived VEGF in normal and polycystic human ovaries. *Am J Pathol*. 2003;162:1881-1893.
13. Viac J, Palacio S, Schmitt D, et al. Expression of vascular endothelial growth factor in normal epidermis, epithelial tumors and cultured keratinocytes. *Arch Dermatol Res*. 1997;289:158-163.
14. Man XY, Yang XH, Cai SQ, et al. Expression and localization of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 in human epidermal appendages: a comparison study by immunofluorescence. *Clin Exp Dermatol*. 2009;34:396-401.
15. Crawford GH, Pelle MT, James WD. Rosacea: I. Etiology, pathogenesis, and subtype classification. *J Am Acad Dermatol*. 2004;51:327-341.
16. Gomaa AH, Yaar M, Eyada MM, et al. Lymphangiogenesis and angiogenesis in non-phymatous rosacea. *J Cutan Pathol*. 2007;34:748-753.
17. Smith JR, Lanier VB, Braziel RM, et al. Expression of vascular endothelial growth factor and its receptors in rosacea. *Br J Ophthalmol*. 2007;91:226-229.
18. Bozduman T, Ersoy Evans S, Karahan S, et al. Genetic risk factors for psoriasis in Turkish population: -1540 C/A, -1512 Ins18, and +405 C/G polymorphisms within the vascular endothelial growth factor gene. *Ann Dermatol*. 2016;28:30-39.
19. Neufeld G, Cohen T, Gengrinovitch S, et al. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J*. 1999;13:9-22.
20. Shibuya M. Vascular endothelial growth factor-dependent and -independent regulation of angiogenesis. *BMB Rep*. 2008;41:278-286.
21. Rogers MS, D'Amato RJ. The effect of genetic diversity on angiogenesis. *Exp Cell Res*. 2006;312:561-574.
22. Fan X, Wu Q2, Li Y, et al. Association of polymorphisms in the vascular endothelial growth factor gene and its serum levels with diabetic retinopathy in Chinese patients with type 2 diabetes: a cross-sectional study. *Chin Med J (Engl)*. 2014;127:651-657.
23. Lv HZ, Lin T, Xia LP, et al. Vascular endothelial growth factor gene polymorphisms and rheumatoid arthritis. *J Investig Med*. 2011;59:593-598.
24. Ma J, Hu W, Zhang P, et al. The association between VEGF +936C/T and -634G/C polymorphisms and Breast cancer susceptibility, tumor growth, and metastases: evidence from 20,728 subjects. *Cancer Invest*. 2015;33:312-317.
25. Chen HL, Liu K. Vascular endothelial growth factor polymorphisms and coronary artery disease: a systemic review and meta-analysis. *Int J Cardiol*. 2014;172:e220-e224.
26. Paradowska-Gorycka A, Pawlik A, Romanowska-Prochnicka K, et al. Relationship between VEGF gene polymorphisms and serum VEGF protein levels in patients with rheumatoid arthritis. *PLoS One*. 2016;11:e0160769.
27. Yoshino S, Cilluffo R, Best PJ, et al. Single nucleotide polymorphisms associated with abnormal coronary microvascular function. *Coron Artery Dis*. 2014;25:281-289.
28. Kamoun M, Houman MH, Hamzaoui A, et al. Vascular endothelial growth factor gene polymorphisms and serum levels in Behcet's disease. *Tissue Antigens*. 2008;72:581-587.
29. Hirakawa S, Fujii S, Kajiya K, et al. Vascular endothelial growth factor promotes sensitivity to ultraviolet B—induced cutaneous photodamage. *Blood*. 2005;105:2392-2399.
30. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med*. 2003;9:669-676.
31. Park JE, Chen HH, Winer J, et al. Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR. *J Biol Chem*. 1994;269:25646-25654.
32. Mustonen T, Alitalo K. Endothelial receptor tyrosine kinases involved in angiogenesis. *J Cell Biol*. 1995;129:895-898.
33. Seetharam L, Gotoh N, Maru Y, et al. A unique signal transduction from FLT tyrosine kinase, a receptor for vascular endothelial growth factor VEGF. *Oncogene*. 1995;10:135-147.
34. Sawano A, Takahashi T, Yamaguchi S, et al. Flt-1 but not KDR/Flk-1 tyrosine kinase is a receptor for placenta growth factor, which is related to vascular endothelial growth factor. *Cell Growth Differ*. 1996;7:213-221.
35. Sawano A, Takahashi T, Yamaguchi S, et al. The phosphorylated 1169-tyrosine containing region of flt-1 kinase (VEGFR-1) is a major binding site for PLCgamma. *Biochem Biophys Res Commun*. 1997;238:487-491.
36. Takahashi T, Ueno H, Shibuya M. VEGF activates protein kinase C-dependent, but Ras-independent Raf-MEK-MAP kinase pathway for DNA synthesis in primary endothelial cells. *Oncogene*. 1999;18:2221-2230.
37. Chappell JC, Taylor SM, Ferrara N, et al. Local guidance of emerging vessel sprouts requires soluble Flt-1. *Dev Cell*. 2009;17:377-386.
38. Topcu-Yilmaz P, Atakan N, Bozkurt B, et al. Determination of tear and serum inflammatory cytokines in patients with rosacea using multiplex bead technology. *Ocul Immunol Inflamm*. 2013;21:351-359.
39. Shin JM, Park JH, Kim HJ, et al. Cigarette smoke extract increases vascular endothelial growth factor production via

TLR4/ROS/MAPKs/NF-kappaB pathway in nasal fibroblast. *Am J Rhinol Allergy*. 2017;31:78-84.

40. Lachgar S, Charvéron M, Gall Y, et al. Inhibitory effects of retinoids on vascular endothelial growth factor production by cultured human skin keratinocytes. *Dermatology*. 1999; 199(suppl 1):25-27.
41. Watson CJ, Webb NJ, Bottomley MJ, et al. Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. *Cytokine*. 2000;12:1232-1235.
42. Stevens A, Soden J, Brenchley PE, et al. Haplotype analysis of the polymorphic human vascular endothelial growth factor gene promoter. *Cancer Res*. 2003;63:812-816.
43. Elfving B, Buttenschøn HN, Foldager L, et al. Depression and BMI influences the serum vascular endothelial growth factor level. *Int J Neuropsychopharmacol*. 2014;17:1409-1417.
44. Kajiya K, Kajiya-Sawane M, Ono T, et al. Identification of an epidermal marker for reddened skin: vascular endothelial growth factor A. *J Dermatol*. 2017;44:836-837.