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ORIGINAL ARTICLE

# 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

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**Background:** Evidence regarding the vascular endothelial growth factor A (VEGFA) as a potent mediator of angiogenesis and inflammation in psoriasis has revealed variations in this gene as surrogate markers of psoriasis. **Objective:** *VEGFA* gene polymorphisms (−1540 C/A, −1512 Ins18, −460 T/C, and +405 C/G) in psoriasis susceptibility in Turkish population were investigated. **Methods:** A total of 200 age, sex and ethnicity-matched psoriatic and healthy individuals were examined for clinical type, response to therapy, serum VEGFA and its receptor levels, genotypes and haplotypes. **Results:** The +405 GG, +405 CG, −1540 CA, and −1512 +Ins18 genotypes conferred a significant risk for developing psoriasis. The C-InsTC haplotype in the controls and C+InsTG, A+InsTC, and A-InsTG haplotypes in psoriatic patients were observed to be significantly high. Increased serum levels of VEGFA were detected in psoriatic patients with the C-InsTC haplotype than that in the controls. The +405 GG genotype was significantly more frequent in psoriatic patients with a positive family history, and the moderate form of psoriasis was more frequent among C+InsTG haplotype carriers than that among the other patients. The +405 GG genotype was found to be more frequent in patients responding to oral retinoids. Serum VEGFR1/FLT1 and VEGFR2/KDR levels were not significantly different when psoriatic patients and controls were stratified based on the risk polymorphic variants. **Conclusion:** *VEGFA* gene +405 GG and CG, −1512+Ins18, and −1540 CA genotypes are associated with an increased risk of psoriasis in Turkish population. The G allele at +405 and an 18-bp insertion at −1512 are primarily the risk factors for psoriasis, and this risk is potentiated by the presence of the A allele at the −1540 locus. **(Ann Dermatol 28(1) 30∼39, 2016)**

-Keywords-
Angiogenesis, Psoriasis, Turkish population, Vascular endothelial growth factor, *VEGFA* gene polymorphisms

## INTRODUCTION

Psoriasis (MIM 177900) is a chronic, recurrent, inflammatory, and hyperproliferative skin disease that results from genetic predetermination in conjunction with environmental factors[1]. Although several genetic loci for psoriasis have been reported, the locus with the strongest effect appears to be the major psoriasis susceptibility 1 (PSORS1) locus within chromosome 6p21[2]. Genes in PSORS1 are believed to play a role in susceptibility to psoriasis and are associated with up to 50% of psoriasis cases[3,4]. The vascular endothelial growth factor A (*VEGFA*) gene is located at the PSORS1 locus, and a substantial body of evidence for VEGFA (MIM 199240) as a potent mediator of angiogenesis and inflammation in psoriasis has been reported[5-8]. VEGFA is overexpressed in psoriatic epidermis, together with its receptors VEGFR1/FLT-1 and VEGFR2/KDR in papillary microvessels[9-13]. In addition, chronic inflammatory lesions resembling human psoriasis

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were reported in the skin of transgenic mice overexpressing VEGFA by Xia et al.[14] VEGFA is thought to play a critical role in the mechanisms underlying vascular changes in psoriasis, and induces psoriasis development by promoting and maintaining immune and inflammatory processes[6,7,15-21]. Therefore, the contribution of VEGFA to the pathogenesis of psoriasis, together with assignment of the *VEGFA* gene to the PSORS1 locus, has indicated *VEGFA* gene variations as candidate markers of psoriasis.

More than 15 single nucleotide polymorphisms (SNPs) of the *VEGFA* gene have been identified within the promoter, 5′-UTR, and exon regions[22-24]. *VEGFA* gene SNPs can influence VEGFA protein production; among them −1540 C/A, −1512 Ins18, −460 T/C, and +405 G/C polymorphisms have been reported as functionally relevant and associated with susceptibility, severity, or course in multiple inflammatory autoimmune diseases[24-29]. The −1540 C/A, −1512 Ins18, and −460 T/C polymorphisms in the promoter region, and +405 G/C in the 5′-UTR, are close to the activator protein-1 sites of the *VEGFA* gene, and are associated with both high and low VEGFA production in diseases with an angiogenic basis[24,27,30-33]. These data suggest that either of these polymorphisms within the regulatory region of the *VEGFA* gene may lead to differences in VEGFA expression between individuals, and that they have a regulatory function. Alternatively, there may be an allelic linkage between these polymorphisms and functional polymorphisms.

To date, only nine case-control studies, two meta-analyses, and two therapeutic response SNP studies of *VEGFA* gene SNPs in psoriasis have been published, and none in Turkish population[34-46]. The results of these previous reports are controversial and associations of *VEGFA* gene SNPs with psoriasis remain unclear. Moreover, eligible case-control studies are required to increase statistical power and provide a more precise evaluation of the association between SNPs and diseases for meta-analysis. However, further well-designed case-control studies are required to evaluate *VEGFA* gene SNPs in relation to psoriasis in different populations for a meta-analytic approach. Previous studies have not assessed the risk of the *VEGFA* gene −1540 C/A (rs699947), −1512 Ins18 (rs699947), −460 T/C (rs833061), and +405 C/G (rs2010963) SNPs together in psoriasis susceptibility. These four *VEGFA* gene SNPs have a high degree of linkage disequilibrium, and were therefore used in this study to investigate psoriasis susceptibility in individuals of a single ethnicity. An association was also analyzed in a subset of psoriatic patients classified by clinical type, disease severity, family history, and response to therapy. Circulating VEGFA together with serum VEGFR1/FLT1 and VEGFR2/KDR levels in relation to risk polymorphic variants and haplotypes were also evaluated. We postulated that the −1540 C/A, −1512 Ins18, −460 T/C, and +405 C/G SNPs contribute to the pathogenesis of psoriasis at the genomic level.

## MATERIALS AND METHODS

### Study population

A total of 100 Caucasian patients with psoriasis who were referred to the Department of Dermatology, Hacettepe University Faculty of Medicine (Ankara, Turkey), were enrolled in this study. A total of 100 sex-matched healthy Caucasian controls of the same ethnicity with no family history of psoriasis were also recruited (Table 1). A detailed interview addressing personal and family history and demographic information was performed in the context of a physical examination. Patients were evaluated based on clinical type (Type I, Type II), disease severity (Psoriasis Area and Severity Index, PASI), family history, and response to therapy. Patients with an age of psoriasis onset below 40 years were considered to have Type I, while those above 40 years were classified as Type II. Psoriasis severity was assessed using PASI (<8=mild, 8~12=moderate, >12=severe). All patients received one of the following therapies: 1) Topical treatment (corticosteroids, vitamin D analogues, retinoids, salicylic acid, calcineurin inhibitors), 2) phototherapy (psoralen ultraviolet A, ultraviolet-B), 3) biological drugs (etanercept, infliximab, adalimumab), 4) immunosuppressive drugs (methotrexate, cyclosporine), or 5) oral retinoids (acitretin). Responses to therapy were objectively assessed by two expert dermatologists using clinical records and direct patient questioning. Response to treatment was determined by a decrease of at least 75% in the pre-treatment PASI after 4 months of therapy. Patient characteristics are summarized in Table 2. Exclusion criteria were psoriatic arthropathy or other inflammatory arthritis, pregnancy, or breastfeeding. The study protocol adhered to the Declaration of Helsinki Guidelines and was approved by the Ethics Committee of Hacettepe University (GO 2013/131-03). Written informed consent was obtained from all patients and controls.

**Table 1.** The age and sex distribution of psoriatic patients and controls

| Variable | Patient (n=100) | Control (n=100) | *p*-value |
|---|---|---|---|
| Age (yr) | 40.7±13.8 | 40.8±13.4 | 0.955 |
| Sex (male/female) | 51/49 | 51/49 | 1.000 |

Values are presented as mean±standard deviation and number.

### *VEGFA* genotyping

Genomic DNA was isolated from whole blood of each individual using Qiagen QIAamp DNA blood mini kit (QIAGEN GmbH, Hilden, Germany). *VEGFA* gene SNPs were determined using polymerase chain reaction (PCR) and restriction endonuclease digestions using specific oligonucleotide primers and restriction enzymes under the appropriate conditions (Table 3). PCR amplification was performed in 50-μl reaction mixtures containing 100 ng of template DNA, 400 μM of each primer, 200 μM of each dNTP, 1×PCR buffer (20 mM Tris-HCl, 50 mM KCl), and 2.5 U of Taq DNA Polymerase (Fermentas Life Sciences, Leon-Rot, Germany). Initial denaturation was performed at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at the indicated temperatures for 1 min, extension at 72°C for 1 min, with a final extension at 72°C for 5 min using an ICycler (Bio-Rad, Hercules, CA, USA). The −1540 C/A and −1512 Ins18 SNPs were amplified under the same PCR conditions. The 18-bp insertion was detectable as a longer PCR product. PCR products were digested with the appropriate restriction enzymes (New England BioLabs Inc., Hertfordshire, UK).

**Table 2.** Characteristics of the psoriatic patients

| Variable | | Patient (n=100) |
|---|---|---|
| Clinical type | Type I (early-onset psoriasis) | 78 |
| | Type II (late-onset psoriasis) | 22 |
| Psoriasis severity (PASI) | Mild (<8) | 22 |
| | Moderate (8~12) | 61 |
| | Severe (>12) | 17 |
| Family history | Positive | 40 |
| | None | 60 |
| Treatment group | Topical treatment | 20 |
| | Phototherapy | 30 |
| | Biological drug | 25 |
| | Immunosuppressive drug | 17 |
| | Oral retinoid | 8 |
| Respond to all therapies | Positive | 73 |
| | None | 27 |

PASI: Psoriasis Area and Severity Index.

### Determination of serum levels of VEGFA, VEGFR1/FLT-1, and VEGFR2/KDR

Serum levels of VEGFA, VEGFR1/FLT-1, and VEGFR2/KDR were analyzed using Boster ELISA (Boster Immunoleader; Boster Biological Technology Co., Ltd., Abingdon, UK). Results were expressed as picograms per milliliter and all parameters were measured in a single run in all samples. Sensitivities of the VEGFA, VEGFR1/FLT-1, and VEGFR2/KDR assays were <1.0 pg/ml, <30.0 pg/ml, and <4.0 pg/ml, respectively.

### Statistical analysis

Statistical analyses were performed using the IBM SPSS for Windows version 21.0 statistical software (IBM Co., Armonk, NY, USA). Haplotype Analysis version 1.04 statistical software was used to determine haplotype distribution. Continuous variables were presented as means±standard deviation and medians, and categorical variables as percentages. An independent sample *t*-test was used to compare the age distribution of the individuals in the control and psoriasis groups. Categorical variables were compared using the chi-squared or Fisher's exact test, as appropriate. Odds ratios and confidence intervals were also calculated. Independent groups were compared by Mann-Whitney U or Kruskal-Wallis test according to the level of VEGFA. The Hardy-Weinberg equilibrium for gen-

**Table 3.** PCR oligonucleotide primer pairs, PCR conditions, corresponding restriction endonucleases, PCR product and restriction fragment sizes

| SNP | Primers sequence | PCR product size (bp) | PCR conditions (annealing temperature, $MgCl_2$) | Restriction enzyme site | Alleles | DNA fragments sizes (bp) |
|---|---|---|---|---|---|---|
| −1540 C/A and −1512Ins18 | F5′-CCC TGG AGC GTT TTG GTTA AA-3′ | 297 (315)* | 64°C, 1 mM | *Bgl*II | C | 297 (315)* |
| | R5′-CCC TTA CCT CCA AGC CCC CT-3′ | | | | A | 197 (215)*,100 |
| −460 T/C | F5′-TGT GCG TGT GGG GTT GAG CG-3′ | 175 | 63°C, 2 mM | *BstU*I | T | 175 |
| | R5′-TAC GTG CGG ACA GGG CCT GA-3′ | | | | C | 155, 20 |
| +405 C/G | F5′-ATT TAT TTT TGC TTG CCA TT-3′ | 304 | 58°C, 3 mM | *BsmF*1 | C | 304 |
| | R5′-GTC TGT CTG TCT GTC CGT CA-3′ | | | | G | 193, 111 |

PCR: polymerase chain reaction, SNP: single nucleotide polymorphism. *PCR product size when the 18 bp insertion at position −1512 is present.

otype frequency was assessed using the chi-squared test.

## RESULTS

### Associations of *VEGFA* genotypes and haplotypes with psoriasis

Genotype frequencies at all loci were in Hardy-Weinberg equilibrium for psoriatic patients and controls ($p>0.05$). Genotype distribution and allele frequencies of the four *VEGFA* gene SNPs in psoriatic patients and controls are shown in Table 4. Significant differences were observed in the genotypic and allelic distribution of −1540 C/A, −1512 Ins18, and +405 C/G SNPs between psoriatic patients and controls.

The +405 GG and CG genotypes were significantly more frequent in psoriatic patients than controls ($p<0.001$). Homozygotes (GG) and heterozygotes (CG) of the +405 C/G SNP increased the risk of developing psoriasis by approximately nine- and seven-fold, respectively (ORs=9.40 and 7.02, respectively, $p<0.001$ for both; Table 5). The G and C alleles were found to be present at high frequencies in psoriatic patients and controls, respectively ($p<0.001$; Table 4).

The 18-bp insertion at the −1512 locus was significantly more frequent in psoriatic patients compared to controls ($p<0.001$). The −1512 Ins18 SNP increased the risk of disease four-fold (OR=4.01, $p<0.001$; Table 5).

The −1540 CA genotype was significantly more frequent in patients with psoriasis compared to controls ($p=0.002$). An approximately two-fold increased risk of psoriasis was observed in the heterozygote genotype of −1540 C/A SNP (OR=2.5, $p=0.004$; Table 5). The A allele frequency was higher in the patient group, whereas the C allele frequency was higher in the control group ($p=0.002$; Table 4).

The genotype and allele distributions of -460 T/C SNP did not significantly differ between patients and controls. Regarding the four loci, all 16 potential haplotypes (combinations of −1540 C/A, −1512 Ins18, −460 T/C, and

**Table 4.** Genotype and allele distributions of four VEGFA SNPs in psoriatic patients and controls

| VEGFA locus | Genotype and alleles | Control (n=100) | Patient (n=100) | $X^2$ | *p*-value |
|---|---|---|---|---|---|
| −1540 C/A | CC | 78 | 57 | 12.741 | 0.002* |
| | AA | - | 3 | | |
| | AC | 22 | 40 | | |
| | C allele | 178 | 154 | 9.373 | 0.002* |
| | A allele | 22 | 46 | | |
| −1512Ins18 | −Ins18 | 72 | 39 | 22.047 | <0.001* |
| | +Ins18 | 28 | 61 | | |
| −460 T/C | TT | 75 | 86 | 3.185 | 0.074 |
| | CT | 25 | 14 | | |
| | CC | - | - | | |
| | T allele | 175 | 186 | 2.841 | 0.092 |
| | C allele | 25 | 14 | | |
| +405 C/G | CC | 73 | 26 | 44.401 | <0.001* |
| | GG | 13 | 40 | | |
| | CG | 14 | 34 | | |
| | C allele | 160 | 86 | 57.819 | <0.001* |
| | G allele | 40 | 114 | | |

VEGFA: vascular endothelial growth factor A, SNPs: single nucleotide polymorphisms. *Statistically significant results.

**Table 5.** Genotypes that are associated with an increased risk of psoriasis

| VEGFA locus | Genotype | Control (n) | Patient (n) | OR (95% CI) | *p*-value (multivariate) |
|---|---|---|---|---|---|
| +405 C/G | GG | 13 | 40 | 9.40 (4.25~20.79) | <0.001* |
| +405 C/G | CG | 14 | 34 | 7.02 (3.24~15.23) | <0.001* |
| −1512Ins18 | + Ins18 | 28 | 61 | 4.10 (2.26~7.47) | <0.001* |
| −1540 C/A | AC | 22 | 40 | 2.51 (1.34~4.68) | 0.004* |
| −460 T/C | CT | 25 | 14 | 0.48 (0.23~1.00) | 0.05 |

VEGFA: vascular endothelial growth factor A, OR: odds ratio, CI: confidence interval. *Statistically significant results.

+405 C/G) are presented in Table 6. There was a significant difference in haplotype distribution; the C-InsTC haplotype was significantly more frequent in controls ($p$ <0.001) and the C+InsTG, A+InsTC, and A-InsTG haplotypes were significantly more frequent in psoriatic patients ($p$<0.001, 0.007, and 0.020, respectively). Haplotype analyses also revealed that the frequencies of the G allele at the +405 locus and 18-bp insertion at the −1512 locus, as well as the A allele at the −1540 locus, were significantly higher in psoriatic patients.

Regarding other combinations of the four SNPs, the most common co-occurrences of genotypes in psoriatic patients were as follows: (+405 GG) homozygous+(−1512 +Ins18) ($p$<0.001); (+405 CG) heterozygous+(−1540 CA) heterozygous+(−1512 +Ins18) ($p$=0.013); (+405 CG) heterozygous+(−1512 +Ins18) ($p$=0.002); (+405 GG) homozygous+(−1540 CA) heterozygous ($p$=0.002); (+405 CG) heterozygous+(−1540 CA) heterozygous ($p$=0.002). Furthermore, the +405 GG genotype was more common in psoriatic patients with a positive family history ($p$<0.05; Table 7). The moderate form of psoriasis (PASI=8~12) was more frequent among C+InsTG haplotype carriers compared to other patients (OR=2.53, $p$=0.016). Although not significant, the C+InsTG haplotype was more frequent in patients with early-onset psoriasis (Type I, 31.4%) compared to those with late-onset psoriasis (Type II, 18.2%) ($p$=0.091).

Differences in genotype frequencies between responders to each treatment groups are shown in Table 8. The +405 GG genotype was more often observed in patients who

**Table 6.** Haplotype frequencies for *VEGFA* gene −1540 C/A, −1512Ins18, −460 T/C and +405 C/G SNPs in psoriatic patients and controls

| Haplotype | Control, n (%) | Patient, n (%) | OR (95% CI) | $p$-value |
|---|---|---|---|---|
| A-InsCC | 7 (3.5) | 1 (0.5) | 0.139 (0.017~1.137) | 0.066 |
| A+InsCC | 2 (1.0) | 1 (0.5) | 0.497 (0.045~5.531) | 0.570 |
| C-InsCC | 3 (1.5) | 0 (0) | 0.246 (0.027~2.223) | 0.212 |
| C+InsCC | 1 (0.5) | 1 (0.5) | 1 (0.062~16.099) | 1.000 |
| A-InsTC | 4 (2.0) | 12 (6.0) | 3.128 (0.991~9.869) | 0.052 |
| A+InsTC | 0 (0) | 14 (7.0) | 16.123 (2.109~123.256) | 0.007* |
| C-InsTC | 99 (49.5) | 13 (6.5) | 0.071 (0.038~0.133) | <0.001* |
| C+InsTC | 44 (22.0) | 44 (22.0) | 1 (0.623~1.605) | 1.000 |
| A-InsCG | 7 (3.5) | 4 (2.0) | 0.563 (0.162~1.953) | 0.365 |
| A+InsCG | 2 (1.0) | 2 (1.0) | 1 (0.139~7.17) | 1.000 |
| C-InsCG | 2 (1.0) | 4 (2.0) | 2.02 (0.366~11.158) | 0.420 |
| C+InsCG | 1 (0.5) | 1 (0.5) | 1 (0.062~16.099) | 1.000 |
| A-InsTG | 0 (0) | 10 (5.0) | 11.576 (1.48~90.523) | 0.020* |
| A+InsTG | 0 (0) | 2 (1.0) | 3.03 (0.313~29.378) | 0.339 |
| C-InsTG | 22 (11.0) | 34 (17) | 1.657 (0.931~2.949) | 0.086 |
| C+InsTG | 6 (3.0) | 57 (28.5) | 12.888 (5.408~30.717) | <0.001* |

VEGFA: vascular endothelial growth factor A, SNPs: single nucleotide polymorphisms, OR: odds ratio, CI: confidence interval. *Statistically significant results.

**Table 7.** Correlation between family history and VEGF genotypes

| VEGFA locus | Genotype | No family history (n=60) | Positive family history (n=40) | $X^2$ | $p$-value |
|---|---|---|---|---|---|
| −1540 C/A | CC | 34 (59.6) | 23 (40.4) | 0.976 | 0.614 |
| | AA | 1 (33.3) | 2 (66.7) | | |
| | AC | 25 (62.5) | 15 (37.5) | | |
| −1512 Ins18 | −Ins18 | 22 (56.4) | 17 (43.6) | 0.343 | 0.558 |
| | +Ins18 | 38 (62.3) | 23 (37.7) | | |
| −460 T/C | TT | 51 (59.3) | 35 (40.7) | 0.003 | 0.953 |
| | CT | 9 (64.3) | 5 (35.7) | | |
| +405 C/G | CC | 18 (69.2) | 8 (30.8) | 6.261 | 0.044* |
| | GG | 18 (45.0) | 22 (55.0) | | |
| | CG | 24 (70.6) | 10 (29.4) | | |

Values are presented as number (%). VEGF: vascular endothelial growth factor. *Statistically significant result.

were responsive to oral retinoids (100%, $p=0.034$). When analyzed in relation to all therapies, the +405 GG and CG genotypes were observed more frequently in patients who respond to all therapies (72.5% and 85.3%, respectively, $p=0.058$) and the C+InsTC haplotype was more frequently observed in patients who did not respond to therapies (31.5%, $p=0.052$).

### Serum VEGFA, VEGFR1/FLT1, and VEGFR2/KDR levels, and *VEGFA* genotypes

Significantly increased serum levels of VEGFA in psoriatic patients with the C-InsTC haplotype were observed when compared to healthy controls with the same haplotype. Mean VEGFA serum levels were 307.6±108.6 pg/ml (range, 194.7~411.2 pg/ml) in psoriatic patients and 148.5±50.2 pg/ml (range, 69.4~213.6 pg/ml) in controls ($p<0.05$). When considered independently of haplotype, serum VEGFA, VEGFR1/FLT1, and VEGFR2/KDR levels did not show significant differences between psoriatic patients and controls (data not shown). We also stratified based on other genotype combinations and did not observe significant differences between psoriatic patients and controls (data not shown).

## DISCUSSION

This study shows that the *VEGFA* gene +405 GG and CG, −1512+Ins18, and −1540 CA genotypes are associated with an increased risk of psoriasis in Turkish population. The +405 GG genotype also carries a significantly increased risk of developing psoriasis that becomes more evident when patients with a positive family history are considered. Patient carriers of the C+InsTG haplotype show an approximately two-fold increased risk of developing moderate (PASI=8~12) in relation to mild (PASI <8) forms of psoriasis. The +405 GG genotype was more often observed in patients who were responsive to oral retinoids. Our findings suggest that the G allele at the +405 locus and 18-bp insertion at the −1512 locus are risk factors for psoriasis, and that this risk is potentiated by the presence of the A allele at the −1540 locus. We found no evidence of an association between the −460 T/C SNP and psoriasis. These findings are consistent with previous reports of a possible critical role of VEGFA in neovascularization and vascular changes as early pathophysiological events in psoriasis[34-42].

To date, only nine case-control studies have explored the linkage between *VEGFA* gene SNPs and the risk of psoriasis[34-42]. The majority of these studies examined possible associations between −460 T/C and +405 C/G SNPs and psoriasis. This is the first study to address the role of the −1540 C/A, −1512 Ins18, −460 T/C, and +405 C/G SNPs together in psoriasis susceptibility, as well as in Turkish population. Among the nine case-control studies, four were conducted in European populations; the other four were in Asian populations, plus one in Canadian population[34-42]. Our results support all previous findings from Asian studies that the +405 GG genotype and G allele at the +405 locus are associated with psoriasis susceptibility[38-41]. In contrast, Young et al.[35,36] and Zablotna et al.[37] reported that the +405 CC genotype is associated with early-onset psoriasis in British and Polish populations,

**Table 8.** Relationship between genotypes and responders to treatment groups

| VEGFA locus | Genotype | Topical treatment | Phototherapy | Biological drug | Immunosuppressive drug | Oral retinoid |
|---|---|---|---|---|---|---|
| −1540 C/A | CC | 6 (46.2) | 11 (68.8) | 10 (71.4) | 8 (100) | 4 (66.7) |
| | AA | ND | 1 (100) | ND | ND | ND |
| | AC | 4 (66.7) | 12 (92.3) | 8 (72.7) | 8 (88.9) | 1 (100) |
| | *p*-value | 0.343 | 0.212 | 1.000 | 1.000 | 0.229 |
| −1512Ins18 | −Ins18 | 5 (71.4) | 14 (82.4) | 4 (57.1) | 6 (100) | 2 (100) |
| | +Ins18 | 5 (38.5) | 10 (76.9) | 14 (77.8) | 10 (90.9) | 3 (50) |
| | *p*-value | 0.350 | 1.000 | 0.355 | 1.000 | 0.464 |
| −460 T/C | TT | 10 (52.6) | 20 (80) | 15 (78.9) | 15 (93.8) | 4 (57.1) |
| | CT | ND | 4 (80) | 3 (50) | 1 (100) | 1 (100) |
| | CC | ND | ND | ND | ND | ND |
| | *p*-value | 1.000 | 1.000 | 0.298 | 1.000 | 1.000 |
| +405 C/G | CC | 2 (33.3) | 6 (66.7) | 2 (50) | 5 (83.3) | ND |
| | GG | 3 (50) | 8 (72.7) | 10 (66.7) | 4 (100) | 4 (100) |
| | CG | 5 (62.5) | 10 (100) | 6 (100) | 7 (100) | 1 (33.3) |
| | *p*-value | 0.553 | 0.059 | 0.082 | 0.333 | 0.034* |

Values are presented as number (%). VEGFA: vascular endothelial growth factor A, ND: not detected. *Statistically significant result.

respectively. Conversely, Barile et al.[34] did not observe an association between the +405 C/G SNP and psoriasis in Italian population. In addition, no association was observed between this SNP and psoriatic arthritis in the Canadian population studied[42]. The results presented here suggest that *VEGFA* genotype distribution differs according to ethnicity; this is in agreement with Asian studies in which strong association has been observed with the +405 GG genotype and G allele and psoriasis risk. In the assessment of haplotype, risk haplotypes always contained the +405 G allele. Furthermore, we found the +405 GG genotype to be more common in psoriatic patients with a positive family history ($p<0.05$), which suggests that this genotype could be used to predict genetic susceptibility of psoriasis development. Wu et al.[40] also reported a strong association between the +405 G allele and risk of psoriasis in Asian populations by meta-analysis, while no significant relationship was detected in a European population. However, heterogeneity in European studies should be noted, and Barile et al.[34] explained this divergence by either a type 1 error in the British study (false-positive association) or by the small number of patients included in the same category in their sample set (type 2 error – false negative). Overall, we observed an approximately nine-fold increased risk of psoriasis with the +405 GG genotype, which was also observed in Asian studies. However, further studies on the +405 G/C genotype in European populations are required.

We demonstrated that the −1512+Ins18 genotype is strongly associated with psoriasis (OR=4.01, $p<0.001$). In addition, the combination of C+InsTG and A+InsTC haplotypes was significantly more frequent in psoriatic patients compared to other haplotypes. To our knowledge, only one other study has explored the −1512+Ins18 genotype in psoriasis, and the results were concordant with our findings that this genotype increases the risk of developing psoriasis[34]. Furthermore, patient carriers of the C+InsTG haplotype in our study showed an approximately two-fold higher risk of developing a moderate compared with a mild form of psoriasis. This finding suggests that together with the +405 G allele, the −1512 Ins18 SNP affects psoriasis phenotype.

We also observed a significant correlation between the −1540 CA genotype and the A allele at the same locus with psoriasis. Barile et al.[34] found that haplotypes containing the −1540 AA genotype showed a significantly increased risk of developing psoriasis that becomes more evident when late-onset disease is considered. The A+InsTC and A-InsTG haplotypes, which carry the A allele, were more common in psoriatic patients than controls in our study.

Regarding the −460 T/C SNP, although the majority of studies have reported an association between haplotypes containing −460 T and psoriasis, we identified no association between this SNP and psoriasis. However, the C+InsTG, A+InsTC, and A-InsTG haplotypes were significantly more frequent in our psoriatic patients.

Furthermore, the stratification of the responsiveness according to each treatment groups by genotype was analyzed. The +405 GG genotype was more frequently observed in patients who were responsive to oral retinoids (100%, $p=0.034$). Genotypes and haplotypes in responders versus non-responders to all therapies were also investigated in our study. There was an increased frequency of the +405 GG and CG genotypes in patients that responded to all therapies (72.5% and 85.3%, respectively) compared to those who did not (27.5% and 14.7%, respectively) ($p=0.058$). Following incubation of oral retinoids in epidermal keratinocytes from patients with the −405 G allele, VEGFA production was significantly downregulated[35]. Although no significant differences in serum VEGFA levels between psoriatic patients and controls were found when stratified by genotype, patients with +405 GG were found to be more responsive to oral retinoids (100%, $p=0.034$). There could therefore be a genotype-dependent likelihood of benefiting from treatment, which seemed to occur for the risk genotypes +405 GG and CG for psoriasis; however, this warrants further investigation. In addition, the C+InsTC haplotype, which contains the −460 T allele, was more frequent in patients that were non-responsive compared to those who were responsive to all therapies in our study (31.5%, $p=0.052$). Results by Young et el.[35] imply that patients with the −460 TT genotype are almost twice as likely to fail therapies as to respond, which is consistent with our results. It is apparent that psoriasis is polygenic, and therefore genetically distinct subsets could be characterized by differential treatment responses.

Previous studies have attempted to determine the mechanisms by which these *VEGFA* gene SNPs affect VEGFA production (Table 9). The +405 C/G SNP is located at the 5′-UTR and lies within a myeloid zinc finger protein (MZF1) binding site[47]. MZF1 is a transcription factor that regulates the transcription of myeloid-specific genes and affects VEGFA transcriptional activity in peripheral blood mononuclear cells[48]. The occurrence of the C allele is thought to reduce the binding specificity of MZF1 and *VEGFA* transcription factors[47]. Moreover, the +405 C/G SNP within the leader sequence of the *VEGFA* gene enhances the activity of internal ribosome entry site B (IRES-B) and promotes the translation of large VEGFA (L-VEGFA) isoforms at an alternative CUG1 codon. These L-VEGFA

isoforms are 205 amino acid residues longer than AUG-initiated VEGFA isoforms and appear to increase the intracellular VEGFA protein pool[8]. Stevens et al.[32] reported the greatest levels of VEGFA protein production for the +405 GG genotype, intermediate levels for GC, and the lowest levels for the CC genotype. Other groups have also reported high VEGFA production with the +405 GG genotype[24], and it is possible that this genotype affects transcriptional activity. The other *VEGFA* gene SNPs at position −1540, −1512, and −460 are located within the promoter region and have been shown to affect the expression of VEGFA. The −1540 SNP was predicted to be the GATA-2 binding site[47]; however, conflicting results have been reported. The −1540 AA and −1512 +Ins18 genotypes are associated with both high and low VEGFA production, while the −460 T/C SNP does not play a role in VEGFA production[24,30-34]. However, when we stratified based on genotype combination, we found no significant differences in serum VEGFA levels between psoriatic patients and controls. No significant differences were observed in serum VEGFR1/FLT1 and VEGFR2/KDR levels between psoriatic patients and controls, nor with the haplotypes in both groups. We observed significantly increased serum levels of VEGFA in psoriatic patients with the C-InsTC haplotype compared to healthy controls with the same haplotype. It should be noted that many conflicting results have been reported on the relationship between *VEGFA* haplotype and VEGFA levels, which may be due to small sample sizes when subclass is used to determine haplotype. Additionally, other confounding factors and many different variables, such as environment, may influence circulating levels of VEGFA in patients. It is also possible that other SNPs within the *VEGFA* gene contribute to gene regulation as this gene is highly polymorphic. Although SNPs that show a significant linkage disequilibrium were chosen in this study, we cannot exclude the possibility that additional SNPs to those investigated here affect serum VEGFA concentrations.

This here is the first case-control study to evaluate the *VEGFA* gene +405 C/G, −460 T/C, −1540 C/A, and −1512 Ins18 SNP genotypes and haplotypes together in psoriasis. Two meta-analyses have provided a more conclusive association between *VEGFA* gene SNPs and psoriasis. In a meta-analysis, case-control studies are identified by searching electronic databases, used to resolve controversial hypotheses by combining results to obtain a more accurate estimation of the effect in question. However, further case-control studies in different populations are required for a more conclusive evaluation of the association between SNPs and diseases for a meta-analytic approach. Wu et al.[40] used meta-analysis to provide evidence for an association between the +405 G allele and psoriasis, which supports our findings. The meta-analysis by Qi et al.[43] supports the hypothesis that the +405 C/G SNP functions as a biological marker of psoriasis. The −460 T allele was associated with genetic susceptibility to psoriasis in the same meta-analysis, however, we did not find a correlation between this SNP and psoriatic patients. Nonetheless, in any meta-analysis, a potential publication bias may exist because studies with negative results are not published. Therefore, statistical power in a meta-analysis can be increased by including case-control studies with both positive and negative results.

In conclusion, the *VEGFA* gene +405 GG and CG, −1512+Ins18, and −1540 CA genotypes are associated with psoriasis in Turkish population. The G allele at the +405 locus and an 18-bp insertion at the −1512 locus are risk factors for psoriasis, and this risk is potentiated by the presence of the A allele at the −1540 locus. The final phenotype of psoriasis may result from the interaction of these psoriasis-susceptible gene polymorphisms with environmental factors, including known risk factors. Based on our results, VEGFA could be a therapeutic target for psoriasis. Identification of high-risk genotypes for psoriasis

**Table 9.** Mechanisms by which *VEGFA* gene SNPs might affect VEGFA production

| VEGFA polymorphism | Location | Role | Effect |
|---|---|---|---|
| +405 C/G | 5′-untranslated | Myeloid zinc finger protein (MZF1) (transcription factor) binding site; enhances the activity of internal ribosome entry site B (IRES-B) and promotes the translation of large VEGFA (L-VEGFA) isoforms at an alternative CUG1 codon | GG genotype associated with the greatest levels of VEGFA |
| −1512 Ins18 and −1540 C/A | Promoter | Effect the expression of VEGFA | Both high and low levels of VEGFA were determined |
| −460 T/C | Promoter | GATA-2-binding site | Neither high nor low levels of VEGFA were determined |

VEGFA: vascular endothelial growth factor A, SNPs: single nucleotide polymorphisms.

may reveal novel treatment options that restore to normal or minimize the proangiogenic phenotype, suggesting a possible role for pharmacogenetics in predicting psoriasis treatment responses. Additional large and well-designed case-control studies in diverse ethnic groups with more detailed individual information that include well-matched controls concerning the effects of other SNPs and haplotypes on psoriasis are required to delineate associations between *VEGFA* gene SNPs with the risk of psoriasis.

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