Investigation of Immunovascular Polymorphisms and Intersections in Psoriasis

Background: Psoriasis is a chronic, inflammatory skin disease. The etiology of the disease is unknown. It is a polygenic and multifactorial disease, which interacts with genetic and environmental factors. Genetic factors (polymorphism/mutation) can alter the immune system and normal physiologically functioning keratinocytes to pathological or predisposition levels.

Aims: We aimed to investigate psoriasis at a different and novel window by searching for vascular and immunological variations and intersections in psoriasis. We investigated the main vascular and hypoxic controlling factors, which are vascular endothelial growth factor (VEGF) and hypoxia inducible factor 1 alpha (HIF-1α), as well as immunological and serotomeric factors, such as TNF-α, IL-10, and 5HT2A, which could connect each other to the pathogenesis of psoriasis.

Subjects and Methods: Nine single nucleotide polymorphisms (SNPs) in five genes were genotyped by mini-array format in 300 subjects: VEGF (rs2010963, rs833061, and rs1570360), HIF-1α (rs11549465), TNF-α (rs361525, rs1799964, and rs1800896), IL-10 (rs1800896), and 5HT2A (rs6311). Results: An association was found between rs1800629 (TNF-α) and Type I psoriasis, and rs833061 (VEGF) and Type II psoriasis. Haplotype analysis suggests that the coexistence of the polymorphisms rs1799964 (TNF-α), rs2010963 (VEGF), rs833061 (VEGF), and rs6311 (5HT2A) may be a protective factor for psoriasis. Conclusion: Our results suggest that the vascular component of the studied vasculo-immunologic variation is more relevant in the pathogenesis of psoriasis.

Key Words: 5HT2A, HIF-1α, IL-10, psoriasis, TNF-α, VEGF

Introduction

Psoriasis is a T-cell-mediated, autoimmune, chronic, and inflammatory skin disease affecting 2–4% of the population worldwide. The etiology of psoriasis is multifactorial, with genetic and immunogenic factors being prominent. However, the exact pathological mechanism, deteriorated pathways, and triggering intersection in the skin are not known. Genetic factors contribute to the clinical significance, the age of onset, type, and severity. Type I (early onset) psoriasis has an age of onset of <40 years and tends to be familial and severe, whereas Type II (late-onset) occurs after the age of 40 years and is more likely to be sporadic.

The characteristics of psoriasis include keratinocyte hyperproliferation, infiltration of inflammatory cells to the dermis and epidermis, incomplete epidermal keratinocyte differentiation, and dilatation of the dermal vessels. TNF-α and HIF-1α are associated with angiogenesis in psoriasis patients. It has also been found that VEGF has a potent mitogenic effect on endothelial cells and plays a role in maintaining angiogenesis in psoriasis. HIF-1α expression was increased in psoriatic lesions and HIF-1α and VEGF expression in keratinocytes showed intracellular colocalization. The effect of VEGF gene +405 C/G (rs2010963), −460 C/T (rs833061), and −1154 A/G (rs1570360) polymorphisms is not clear. Expression of HIF-1α in epidermal keratinocytes is colocalized with VEGF 3. HIF-1α is overexpressed in psoriatic patients. The HIF-1α −1772 C/T (rs11549465) polymorphism is related with overexpression. Serum TNF levels increase in psoriasis patients. Although TNF-α −238 A/G (rs361525) versus −1031 C/T (rs1799964) polymorphisms were found to be risk factors for psoriasis, the −308 A/G (rs1800629) polymorphism was found to be a protective factor. In psoriasis patients, serum TNF levels increase, whereas IL-10 levels decrease.
IL-10 is an anti-inflammatory and immunosuppressive cytokine, which exhibits pleiotropic effects and is important for the regulation of the immune response in psoriasis. Polymorphisms in the promoter region of the IL-10 gene alter the level of IL-10 expression; the -1082 A/G (rs1800896) polymorphism was associated with moderate expression and late-onset disease.\[10\]

The serotonergic system plays an important role in psoriasis. It is known that the amount of serotonin in patients’ platelets is low.\[11\] However, the expression of the 5HT2A receptor in psoriatic skin is significantly elevated.\[12\] The polymorphism rs6311 of the HT2A gene, which is located in the binding region of the Th1/E47 transcription factor, has been associated with late onset of psoriasis.\[13\]

Vascular changes in psoriasis are quite obvious and important. Vascular changes are mainly controlled by the immunologic factors TNF-\(\alpha\) and IL-10, the hypoxic factor HIF-1\(\alpha\), and the serotonergic factor 5HT2A. We aimed to investigate psoriasis in a novel way by researching vasculo-imunological perspectives.

Subjects and Methods

Patients and controls

This study included 150 clinically diagnosed Turkish psoriasis patients recruited from September 2015 to April 2016, admitted to the Department of Dermatology, and 150 Turkish healthy volunteers as a control group. The 150 psoriasis patients included 92 female and 58 male subjects, and the 150 controls were comprised of 108 females and 42 males. The mean age ± SD for patients and controls were 43.48 ± 16.59 and 35.34 ± 13.46, respectively. Patients were evaluated by Psoriasis area and severity index (PASI) scoring,\[14\] as well as clinical and demographic parameters. Written informed consent was obtained from patients and volunteers prior to their inclusion in this work.

DNA isolation and genotyping

Blood samples in EDTA were obtained from patients and controls. Genomic DNA was isolated from whole blood samples using the Pure Link\textsuperscript{TM} Genomic DNA Mini Kit (Invitrogen). DNA was quantified using the Nanodrop2000 spectrophotometer. All samples were standardized to a concentration of 10 ng/\(\mu l\).

Genotyping was performed using the Taq-Man\textsuperscript{TM} real-time PCR with TaqMan Assays (Applied Biosystems\textsuperscript{TM}). VEGF (rs2010963, rs833061, and rs1570360), HIF-1\(\alpha\) (rs11549465), TNF-\(\alpha\) (rs361525, rs1799964, and rs1800629), IL-10 (rs1800896), and 5HT2A (rs6311) SNPs were genotyped. A final volume of 20 \(\mu l\), consisting of 10 \(\mu l\) TaqMan\textsuperscript{TM} Genotyping Master Mix (Applied Biosystems\textsuperscript{TM}) and 2 \(\mu l\) genomic DNA (20 ng), was dispensed into each well. The TaqMan\textsuperscript{TM} Genotyping Master Mix (Applied Biosystems\textsuperscript{TM}) contains ROX dye, which serves as a passive reference dye to normalize signals and ensure data integrity. The qPCR was run on the StepOne-Plus (Applied Biosystems\textsuperscript{TM}) with the following thermal cycling conditions: pre-PCR hold at 60°C for 30 s, initiation at 95°C for 10 min for initial denaturation and enzyme activation, followed by 50 cycles of 92°C for 15 s, then 60°C for 90 s, and a final step at 60°C for 30 s.

The TaqMan Predesigned Genotyping Assay contains two primers for amplifying the sequence of interest and two TaqMan\textsuperscript{®} minor-groove binding probes for detecting alleles. Each probe was labeled with a VIC\textsuperscript{®} dye-labeled probe and FAM\textsuperscript{TM} dye-labeled probe.

Statistics

Relationships between demographic, clinical, and genotype parameters of the subjects were tested. Chi-square test was applied when relations between categorical variables were determined. For statistical tests, significance was defined as \(P < 0.05\). SHEsis was used for haplotype analysis.\[15\]

Results

Genotypes and allele frequencies

In this study, 150 psoriasis patients and 150 controls were evaluated for 9 SNPs in 5 genes: TNF-\(\alpha\), VEGF, HIF-1\(\alpha\), IL-10, and 5HT2A. We did not find any statistically significant differences in the frequency of the evaluated alleles and genotypes between psoriasis patients and controls [Table 1].

According to the onset of psoriasis, TNF-\(\alpha\) rs1800629 was found to be associated with Type I psoriasis (\(P = 0.010\)) and VEGF rs833061 was associated with Type II psoriasis (\(P = 0.035\)) [Table 2].

PASI is a reliable and reproducible scoring method in adult psoriasis. The severity of the disease is determined according to the PASI score. In this study, PASI 1–5 was considered mild, 5–10 medium, and > 10 severe. There was no significant difference between the PASI score and patient genotypes.

We also considered the question of whether the SNPs were important when patients were considered as having “sporadic” disease (without any family history of psoriasis) or “familial” disease (with a family history of psoriasis). VEGF rs1570360 was found to be associated with susceptibility to sporadic psoriasis [Table 3].

As is already known in the literature, we confirmed that Type I psoriasis is associated with a familial history [Table 4].

Haplotype analysis

Haplotype analysis was conducted for nine SNPs: rs361525, rs1799964, rs1800629, rs2010963, rs833061, rs1570360, rs11549465, rs1800896, and rs6311. The
Table 1: Genotype and allele frequencies of psoriasis patient and control groups

| SNP        | Genotype/Allele | Psoriasis patients | Control group | P     |
|------------|-----------------|--------------------|---------------|-------|
|            | Genotype/Allele | n   | %    | n   | %    |       |
| TNF-α      | GG              | 135 | 90.0 | 130 | 86.7 | 0.306 |
| rs361525   | GA              | 15  | 10.0 | 18  | 12.0 |       |
| (−238A/G)  | AA              | 0   | 0.0  | 2   | 1.3  |       |
|            | G               | 285 | 95.0 | 278 | 92.7 | 0.234 |
|            | A (minor allele)| 15  | 5.0  | 22  | 7.3  |       |
| TNF-α      | CC              | 8   | 5.3  | 8   | 5.3  | 0.759 |
| rs1799964  | CT              | 54  | 36.0 | 48  | 32.0 |       |
| (−1031 T/C)| TT              | 88  | 58.7 | 94  | 62.7 |       |
|            | C               | 64  | 21.3 | 70  | 23.3 | 0.556 |
|            | T (minor allele)| 236 | 78.8 | 230 | 76.7 |       |
| TNF-α      | GA              | 129 | 86.0 | 122 | 81.3 | 0.307 |
| rs1800629  | AA              | 20  | 13.3 | 24  | 16.0 |       |
| (−308 A/G) | G               | 1    | 0.7  | 4   | 2.7  |       |
| VEGF       | GG              | 52  | 34.7 | 55  | 36.7 | 0.034 |
| rs2010963  | GC              | 20  | 13.3 | 19  | 12.6 |       |
| (+405 C/G) | A               | 114 | 88.7 | 117 | 76.3 |       |
|            | C (minor allele)| 20  | 13.3 | 24  | 16.0 |       |
| VEGF       | GG              | 52  | 34.7 | 55  | 36.7 | 0.034 |
| rs833061   | GC              | 20  | 13.3 | 19  | 12.6 |       |
| (−460 T/C) | C               | 114 | 88.7 | 117 | 76.3 |       |
|            | T (minor allele)| 20  | 13.3 | 24  | 16.0 |       |
| VEGF       | GG              | 72  | 48.0 | 78  | 52.0 | 0.127 |
| rs1570360  | GA              | 56  | 37.3 | 61  | 40.7 |       |
| (−1154 A/G)| AA              | 22  | 14.7 | 11  | 7.3  |       |
|            | G               | 217 | 72.3 | 200 | 66.7 | 0.131 |
|            | A (minor allele)| 83  | 27.7 | 100 | 33.3 |       |
| HIF-1α     | CC              | 109 | 7.7  | 123 | 82.0 | 0.155 |
| rs11549465 | CT              | 38  | 25.3 | 25  | 16.7 |       |
| (−1772 T/C)| TT              | 3   | 2.0  | 2   | 1.3  |       |
|            | C               | 271 | 90.3 | 256 | 85.3 | 0.061 |
|            | T (minor allele)| 29  | 9.7  | 44  | 14.7 |       |
| IL-10      | TT              | 57  | 38.0 | 66  | 44.0 | 0.503 |
| rs1800896  | TC              | 78  | 52.0 | 68  | 45.3 |       |
| (−1082 C/T)| CC              | 15  | 10.0 | 16  | 10.7 |       |
|            | C (minor allele)| 100 | 33.3 | 108 | 36.0 | 0.492 |
|            | T               | 200 | 66.7 | 192 | 64.0 |       |
| 5HT2A      | CC              | 50  | 33.3 | 51  | 34.0 | 0.562 |
| rs6311     | CT              | 69  | 46.0 | 75  | 50.0 |       |
| (−1438 T/C)| TT              | 31  | 20.7 | 24  | 16.0 |       |
|            | C               | 173 | 57.7 | 169 | 56.3 | 0.741 |
|            | T (minor allele)| 127 | 42.3 | 131 | 43.7 |       |

frequencies of the two haplotypes “G T G C T G T T T” (P = 0.042) and “G T G G T G T C T C” (P = 0.019) were significantly higher in the control group than in the case group. This suggests that the two haplotypes may be protective against the disease.

**Discussion**

This study evaluated the relationship between nine SNPs in the five genes TNF-α, VEGF, HIF-1α, IL-10, and 5HT2C, as genetic risk factors, which control immunologic and vascular parts of psoriasis, and clinical parameters in a cohort from Denizli region.

Gallo *et al.*[7] and Cabaleiro *et al.*[16] indicated that TNF-α promoter genotypes −238 GG and −1031 TT have been reported to play an important role in the pathogenesis of the disease and both polymorphisms are known to increase psoriasis risk, but no significance was found for −308 G/C. Jia *et al.*[9] and Zhuang *et al.*[6] reported that the −238 A allele was associated with an increased risk of psoriasis and also had an effect in early-onset disease, but the −308 A allele was associated with a reduced risk of psoriasis. Accordingly, Popadic *et al.*[17] reported that the TNF-α −308 G allele was detected at a higher frequency in patients compared to controls and that a lack of the G allele was associated with lower psoriasis severity. Karam *et al.*[14] indicated that there is a significant correlation between TNF-α 308 G and IL-10 1082 G alleles in terms of psoriasis. In our study, no significant difference was found between the patients and controls for the TNF-α −238 A/G, −1031 C/T, and −308 A/G polymorphisms. We found only that TNF-α −308 A allele is associated with Type I psoriasis (P = 0.038).

In addition, the VEGF +405 C allele has been found to be associated with inflammatory or neoplastic diseases.[18] Qi *et al.*[14] pointed out that the +405 G and −460 A alleles might have a correlation with a reduced risk of psoriasis in Asians, whereas the −1154 A allele was associated with significant protection from psoriasis among Caucasians. On the other hand, Carlstro[5] indicated that there is no association between +405 G and −460 C alleles and psoriasis susceptibility in Swedish patients. The VEGF −1154 A allele was found at a frequency of 27.7% in patients and 33.3% in controls; it is remarkable that both protective haplotypes include the −460 C allele.

The HIF-1α −1772 T allele is associated with gene overexpression and is known to be a risk factor in many types of cancer.[19] The HIF-1α protein is high in the serum of psoriasis patients.[20] Expression of HIF-1α in epidermal keratinocytes is colocalized with VEGF.[1] Although the −1772 T allele was found in 9.7% of patients, which is 1.5-fold lower than in controls (14.7%), we could not find any significance (P = 0.061). It might be a more significant result if a larger patient population was examined, but there have been no papers in the literature regarding HIF-1α polymorphisms to date.

IL-10 polymorphisms are disease modifiers but not risk factors; however, they may also influence the clinical
course of the disease. Wongpiyabovorn et al.\textsuperscript{[10]} and Indhumathi et al.\textsuperscript{[21]} reported that −1082 A/G was not associated with a risk of psoriasis in the Thai and South Indian Tamil populations. In our study, we also could not find any association between −1082 A/G and psoriasis.

Ronpirin et al.\textsuperscript{[13]} stated that the polymorphism located in the promoter region of the 5HT2A gene, the −1438 A allele, has been associated with late-onset psoriasis in the Thai population. However, Prieto-Pérez et al.\textsuperscript{[22]} found an association between the −1438 A allele and psoriasis susceptibility in Caucasians and a worse response to the anti-TNF drug. In our study, the 5HT2A −1438 A allele was not directly associated with the disease (\(P > 0.05\)); however, according to haplotype analysis, it could be protective when linked to the “G T G C T G C T T” haplotype.

All this information reaffirms the significance of the polymorphisms, providing clues about the pathologic mechanism of psoriasis. Moreover, it emphasizes the usefulness of haplotypes, which seem to have lost favor in recent years, by empowering the sense of the single SNP’s importance.

### Table 2: Onset of psoriasis versus SNP

| SNP       | Allele | Type I psoriasis | Type II psoriasis | \(P\) |
|-----------|--------|------------------|-------------------|------|
| rs1800629 | GG     | 80 80.8          | 49 96.1           | 0.038|
|           | GA     | 18 18.2          | 2 3.9             |      |
|           | AA     | 1 1.0            | 0 0               |      |
|           | G      | 178 89.9         | 100 98.0          | 0.010|
|           | A      | 20 10.1          | 2 2.0             |      |
| rs833061  | CC     | 23 23.2          | 4 7.8             | 0.05 |
|           | CT     | 47 47.5          | 27 52.9           |      |
|           | TT     | 29 29.3          | 20 39.2           |      |
|           | C      | 93 47.0          | 35 34.3           | 0.035|
| T         | 105 53.0 | 67 65.7   |          |      |

### Conclusion

Our results suggest that the vascular component of the studied vasculo-immunologic variation is more effective in psoriasis. Larger group of subjects and new data sets needed to support these findings.

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### Conflicts of interest

There are no conflicts of interest.

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