Investigating the Role of IL-17A gene rs2275913 Variant in Rosacea: *In Silico* Analysis Suggests Further Studies

Rosacea'da IL-17A geni rs2275913 Varyantının Rolünün Araştırılması: *In Silico* Analiz Önerileri

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Öz

Vücudun patojenlere karşı bağışıklık tepkisinin önemli bir bileşeni olan İnterlökin-17 (IL-17), çeşitli inflamatuar süreçlerde rol oynar. Özellikle rosacea hastalarının derisi kronik inflamasyon sergiler ve IL-17'nin ek proinflamatuar kemokinler ve sitokinlerin üretimini indüklediği bilinmektedir. Bu inflamatuar kaskad, genişlemiş kan damarları, bağışıklık hücresi infiltrasyonu ve papül ve püstüllerin gelişimi dahil olmak üzere rosacea'nın ayırt edici özelliklerine katkıda bulunabilir. Çalışmada IL-17A genindeki spesifik bir genetik varyasyonun (-197 G>A; rs2275913) rosacea duyarlılığı ile ilişkili olup olmadığının incelenmesi amaçlandı. IL-17A varyantını ve rosacea riskini 31 sağlıklı bireyde ve rosacealı 25 bireyde karşılaştırdık. IL-17A varyantının genotiplemesi PCR-RFLP yöntemi kullanılarak yapıldı. Genotip ve alel frekans dağılımları gruplar arasında ki-kare testi ($\chi 2$) kullanılarak karşılaştırıldı. Ek olarak, web tabanlı araçlar kullanılarak IL-17A geninin gen ontolojisi (GO) analizi de gösterilmektedir. Bu çalışmada rs2275913 polimorfizmi ile rosacea duyarlılığı arasında anlamlı bir ilişki gözlenmedi (p=0.124), ancak *in silico* analizi IL-17A gen etkileşim ağının hastalıkta rol oynayabileceğini düşündürdü. IL-17A'nın ve ilgili genlerin, özellikle de bağışıklık savunması ve inflamatuar süreçlerdeki düzenlenmesindeki kritik işlevi göz önüne alındığında, rosacea gelişimi üzerindeki potansiyel etkisinin daha fazla araştırılması gerekmektedir.

Anahtar Kelimeler: IL-17A, İnflamatuar Sitokinler, Gen Polimorfizmi, Rosacea

Abstract

Interleukine-17 (IL-17), a crucial component of the body's immune response against pathogens, is also implicated in various inflammatory processes. Notably, the skin of rosacea patients exhibits chronic inflammation, and IL-17 is known to induce the production of additional pro-inflammatory chemokines and cytokines. This inflammatory cascade can contribute to the hallmark features of rosacea, including dilated blood vessels, immune cell infiltration, and the development of papules and pustules. The study aimed to examine whether a specific genetic variation in the IL-17A gene (-197 G>A; rs2275913) is associated with rosacea susceptibility. We compared the IL-17A variant and rosacea risk in 31 healthy individuals and 25 with rosacea. Genotyping of the IL-17A variant was performed using the PCR-RFLP method. Genotype and allele frequency distributions were compared across groups using the chi-square test ($\chi 2$). Additionally, gene ontology (GO) analysis of the IL-17A gene using web-based tools is also demonstrated. No significant association between the rs2275913 polymorphism and rosacea susceptibility was observed in this study (p=0.124) but *in silico* analysis suggested that the IL-17A gene interaction network might play a role in the disease. Given its critical function in regulating IL-17A and related genes, particularly in immune defense and inflammatory processes, further investigation into its potential influence on rosacea development is required.

Keywords: IL-17A, Inflammatory Cytokines, Gene Polymorphism, Rosacea

Introduction

Rosacea is a chronic skin condition distinguished by persistent redness, typically concentrated in the central region of the face. Predominantly affecting individuals aged 30 to 60, this dermatological disorder poses unique challenges due to its prolonged nature (1). While a definitive cure for this skin disorder remains elusive, there exist specific treatment modalities that aim to mitigate its effects. The global prevalence of rosacea currently stands at approximately 5%, and projections indicate a potential rise in this rate in the years to come (2). While the exact triggers of rosacea remain elusive, there's mounting evidence pointing towards an intricate interplay of genetics and environmental influences (3,4). Although the pathophysiology of rosacea is multifactorial, studies conducted in recent years emphasize the importance of innate and acquired immunity. The expression of genes facilitated by TH17, which encode cytokines such as IL-17A, IL22, IL6, IL20, and chemokine CCL20, is believed to be a significant contributing factor in the intricate landscape of rosacea development (5,6). IL17A is produced by CD4+ T helper (TH) cells and is an important pro-inflammatory cytokine in triggering the immune

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response. Since IL-17 is one of the triggers of the inflammatory process, it contributes to the pathophysiology of rosacea (7).

Additionally, studies have elucidated the angiogenic properties of IL-17, expanding our understanding of its multifaceted role in the pathogenesis of rosacea. In particular, while CD4+ TH cells typically dominate immune cell infiltrates, rosacea presents a distinct TH1/TH17 polarization pattern that distinguishes it from conditions such as acne and vulgaris (8). Although the connection between the active roles of IL-17A in the inflammatory response and the inflammation process in the pathogenesis of rosacea has been elucidated, more findings are needed about the contribution of IL-17A gene variants to the pathogenesis of rosacea. The study aimed to investigate the potential effect of the IL-17A gene rs2275913 variant on rosacea disease.

Material and Method

Study Groups

The study sample group was created using convenience sampling. Our study included 25 adult individuals (25 patients) who applied to Muğla Sıtkı Koçman University, Department of Dermatology and Venereology, were diagnosed with rosacea disease, were over 18 years of age, had cognitive ability, and did not have a serious immune disease. The healthy control group included 31 adults over the age of 18 (31 controls) who had not previously been diagnosed with any skin or venereal disease or serious chronic disease. The study did not include infection, heart failure, cancer, or pregnant and haematological patients. This research adhered to the ethical principles outlined in the Declaration of Helsinki and received prior approval from the Muğla Sıtkı Kocman University Faculty of Medicine Ethics Committee. Each participant actively provided written informed consent prior to their involvement in the study.

Molecular Analyses

Blood samples from participants for routine testing were stored at -40°C for DNA extraction. DNA extraction was employed using the Hibrigen Blood DNA Isolation Kit (Hibrigen Biotechnology R&D Industry and Trade Inc., Turkey). The rs2275913 polymorphism within the IL-17A gene was genotyped using PCR-RFLP analysis. The PCR reaction, performed in a 25-µL volume, included 100 ng of genomic DNA and 2X Taq Master Mix (Hibrigen Biotechnology R&D Industry and Trade Inc., Turkey). The specific primer sequences and the PCR and RFLP conditions are provided in Table 1.

Statistical analysis

Categorical data analysis was performed using Yates' corrected chi-square test and Fisher Freeman-Halton tests in SPSS (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The percentage (%) and n (frequency) represent the category values. A p-value of less than 0.05 showed statistical significance for the results.

The power of the study was calculated using posterior power analysis and "G-Power-3.1.9.4" program. The power of the study, which included a total of 56 patients with a medium effect size, was determined to be 0.72.

In silico analyses

We leveraged the STRING database to elucidate potential interactions between IL-17A and the network proteins (https://string-db.org/). This tool provides information about how proteins interact with each other, both directly and indirectly, revealing not only their physical connections but also how they work together. Furthermore, we utilized DisGeNET (http://www.disgenet.org/home) to delve into gene-disease associations, specifically focusing on genes linked to rosacea (C0035854). By mining this curated database, we aimed to identify genes potentially involved in the pathogenesis of this skin condition.

Results

Molecular analyses

DNA samples taken from 25 patients and 31 healthy individuals over the age of 18 were analysed for IL-17A gene rs2275913 variant. The genotype and allele distributions of the IL-17A gene rs2275913 variant are shown in Table 2 and gel visualisation is shown in Figure 1.

Table 1. PCR and RFLP conditions used for polymorphisms of IL-17A gene

PCR conditions of the IL-17A gene rs2275913 variant				
Gene	Polymorphism	Primers	Temperature of annealing	Product size
IL17A	rs2275913 (-196 G>A)	P1 P2	65 °C	445 bp
RFLP conditions of the IL-17A gene rs2275913 variant				
Gene	Polymorphism	Restriction enzyme	Digestion conditions	Restriction fragment sizes
IL17A	rs2275913 (-196 G>A)	*Eco*NI	37°C, 16-18h	G allele: 445 bp A allele:148 and 297 bp

R: 5'-GCATAACTCTTCTGGCAGCTGTA-3, F: 5'-GTATTTCTGGACCGTGGGCA-3'

The control group exhibited genotype frequencies consistent with Hardy-Weinberg equilibrium (p=0.138). For the rs2275913 polymorphism, the distribution of GG, AG and AA genotypes was 12%, 4% and 84% in the case group, and 3.2%, 19.4% and 77.4% in the control group, respectively. We have detected no significant difference in the genotype frequencies and allele frequency the between two groups (p>0.999) (Table 2).

Table 2. Distribution of IL-17A genotypes and allele frequencies in Cases and Healthy controls

	Healthy controls *n* (%)	Cases *n* (%)	*p*
Genotype rs2275913 (-197 G>A)			
AA	24 (77.4)	21 (84.0)	
GG	1 (3.2)	3 (12.0)	0.138
AG	6 (19.4)	1 (4.0)	
Allele rs2275913 (-197 G>A)			
G	54 (87.1)	43 (86.0)	>0.999
A	8 (12.9)	7 (14.0)	

In silico Analyses

We obtained functional enrichment descriptions for potential STRING networks using the interactions IL17A. STRING reported pathways related to recognized networks, drawing from the Gene Ontology and Reactome Pathways databases (Table 3). The results of positive regulation of plasma cell differentiation, IL17 receptor activity and CD163 mediating an anti-inflammatory response ranked first. The prominent results were related to inflammation, consistent with the pathogenesis of rosacea. Among the genes annotated in these results were IL2, IL10, IL17RA, IL17RC and IL6 genes. A total of 41 genes associated with rosacea were identified in the DisGeNET database. In the analysis results, CAMP, TRPV4, HLA-DRA, IL17A, BTNL2, PSG2, OCA2, NHS, HLA-DRB AND CD79A genes were among the top 10 genes.

Discussion

Rosacea is a chronic inflammatory skin disorder characterized by persistent facial erythema. Its pathogenesis involves a complex interplay of genetic and environmental factors, culminating in the activation of various immune cell types, including keratinocytes, mast cells, endothelial cells, and T lymphocytes (9). Among these, Th17 cells play a pivotal role, releasing pro-inflammatory cytokines like IL-17A, which contribute to the characteristic inflammation observed in rosacea lesions (10,11). This study aimed to explore the potential association between the rs2275913 polymorphism within the IL-17A gene and rosacea susceptibility. While our analysis did not reveal a statistically significant difference in genotype or allele frequencies between rosacea patients and healthy controls, the findings warrant further discussion in the context of existing literature.

Different studies highlight the important role of IL-17A in the pathogenesis of various inflammatory skin disorders, including psoriasis, ankylosing spondylitis, and rheumatoid arthritis. It has been suggested that inhibiting IL-17 may be beneficial in the treatment of chronic inflammatory diseases such as psoriasis, ankylosing spondylitis, and rheumatoid arthritis (6). In a mouse model of imiquimod-induced psoriasis, the pro-inflammatory cytokine IL-17 was identified as a regulator of IL-25, a protein highly expressed in psoriatic skin lesions (12). Increased protein levels of IL-17F, IL-17A and IL-17C in psoriatic skin lesions demonstrated that, in addition to IL-17F, IL-17A and IL-17C also plays a potential role in pathogenesis (13). In the management of psoriasis, secukinumab has demonstrated its ability to provide long-term symptom relief by selectively neutralizing IL-17A, the primary inflammatory cytokine in this autoimmune disorder (14). Pityriasis rubra pilaris (PRP), a rare acquired inflammatory skin condition, is associated with increased levels of Th17 and Th1 cytokines, including IL-17A, IL-6, TNF, IL-22, IL-12, IL-23, and IL-17F (15). Quantitative analysis in lesional skin of Systemic Sclerosis revealed higher mRNA expression of Th17 cytokines, including IL-13, IL-17A, IL-26 and IL-22 compared to healthy controls (16).

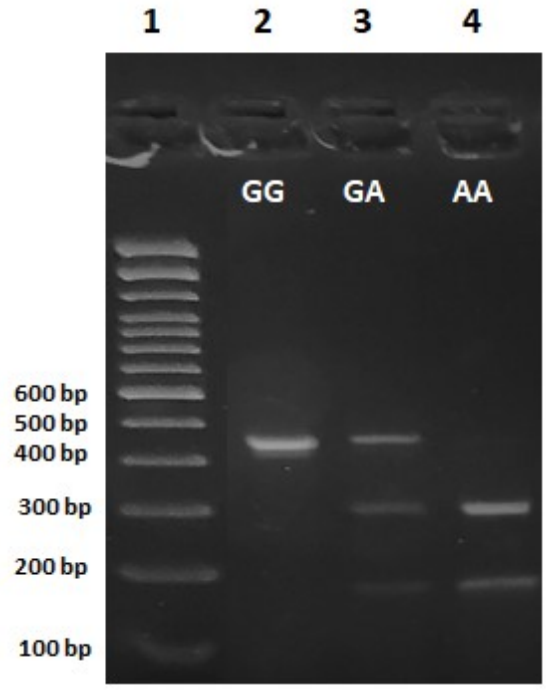
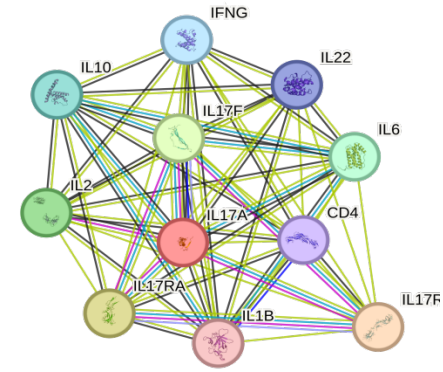
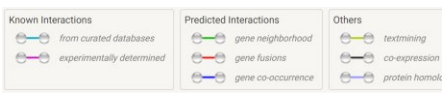


Figure 1. Polymerase chain reaction-restriction fragment length polymorphism analysis for genotyping of IL-17A rs2275913 variation. Lane 1 is a 100 bp DNA marker, lane 2 is GG genotype, lane 3 is GA genotype; lane 4 is AA genotype.

Table 3. Functional enrichments of networks based on IL-17A interactions

Network	Database	Description	Strength[1]	FDR[2]
 (number of nodes: 11, number of edges: 54, average node degree: 9.82, avg. local clustering coefficient: 0.982, expected number of edges: 23, PPI enrichment p-value: 3.24e-08)	Gene Ontology-Biological Process	1# GO:1900100 Positive regulation of plasma cell differentiation	3.08	0.00040
		2# GO:0060559 Positive regulation of calcidiol 1-monooxygenase activity	3.08	0.00040
		3# GO:2000340 positive regulation of chemokine (C-X-C motif) ligand 1 production	3.03	2.77e-06
		4# GO: 0032747 Positive regulation of interleukin-23 production	2.89	5.06e-06
		5# GO:0097400 Interleukin-17-mediated signaling pathway	2.78	7.95e-06
	Gene Ontology-Molecular Function	1# GO:0030368 Interleukin-17 receptor activity	2.65	0.0070
		2# GO:0005125 Interleukin-17 receptor activity	1.79	3.63e-10
		3# GO:0004896 Cytokine receptor activity	1.75	0.0088
		4# GO:0070851 Growth factor receptor binding	1.72	0.00053
		5# GO:0005126 Cytokine receptor binding	1.67	4.60e-08
	Reactome Pathways	1# HSA-9662834 - CD163 mediating an anti-inflammatory response	2.6	0.0032
		2# HSA-8877330 RUNX1 and FOXP3 control the development of regulatory T lymphocytes (Tregs)	2.55	0.0033
		3# HSA-6783783 Interleukin-10 signaling	2.08	0.00056
		4# HSA-448424 Interleukin-17 signaling	2.02	2.13e-05
		5# HSA-8950505 Gene and protein expression by JAK-STAT signaling after Interleukin-12 stimulation	1.99	0.0296



[1]**Strength:** Log10(observed / expected). This measure describes how large the enrichment effect is. It's the ratio between i) the number of proteins in your network that are annotated with a term and ii) the number of proteins that we expect to be annotated with this term in a random network of the same size.
[2]**False Discovery Rate:** This measure describes how significant the enrichment is. Shown are p-values corrected for multiple testing within each category using the Benjamini–Hochberg procedure.

There are studies investigating the relationship of various variants of the IL-17A gene with different dermatological diseases. It has been reported that the IL-17A rs2275913 variant does not affect the course of atopic dermatitis (17). The -152 G/A IL-17A variant AA genotype has been found to increase the risk of developing atopic dermatitis (18). It has been determined that AA+GA genotypes of the IL-17A (rs10484879) variant increase fungal growth and psoriasis susceptibility (19). A similar study determined that the IL-17A (rs10484879) G/T variant was effective in the pathogenesis of psoriasis in a North Indian population (20). Another study found no association between IL-17A rs4711998 and IL-17A rs2275913 variants and the development of vitiligo (21).

While the exact role of IL-17A in rosacea pathogenesis remains to be fully elucidated, the current understanding suggests its involvement in the inflammatory cascade. Further research is warranted to explore the functional significance of specific IL-17A variants and their potential interactions with other genetic and environmental factors in rosacea development. Additionally, investigating the broader immunological landscape, including Th17 cell subsets and other cytokine profiles, might provide deeper insights into the disease mechanisms.

The utilization of STRING and DisGeNET databases provided valuable insights into potential protein-protein interactions and gene-disease associations relevant to rosacea. Notably, genes like CAMP, TRPV4, and IL17A emerged as potential players in the rosacea network, warranting further investigation. These findings pave the way for future studies to delve deeper into the molecular mechanisms underlying rosacea and identify potential therapeutic targets.

In our study, the small number of participants is a significant limitation. Furthermore, the investigation was restricted to a single polymorphism within the IL-17A gene (rs2275913). Evaluating the influence of additional polymorphisms within this gene may offer more comprehensive insights into the potential role of IL-17A in rosacea pathogenesis.

Conclusion

This study investigated the potential association between the IL-17A gene variant rs2275913 and rosacea susceptibility. While *in silico* analyses revealed functional enrichment related to rosacea pathways and identified genes potentially involved in the disease, no significant differences in genotype or allele frequencies were observed between the rosacea patient and control groups. These findings suggest that the IL-17A rs2275913 variant may not be a direct contributor to rosacea development. However, further research with larger sample sizes and exploring additional IL-17A variants or functional studies might be necessary to definitively rule out its involvement.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethics Committee Approval: The study protocol was approved by Muğla Sıtkı Koçman University Faculty of Medicine Medical Ethics Committee (28/07/2021 dated and 16/II numbered decision). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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