Sabuncuoglu Serefeddin Health Sciences (SSHS)

ISSN: 2667-6338, 2023/Vol.5:2/29-38

ANALYSIS OF CTLA 4 GENE +49A/G AND CT60 A/G POLYMORPHISMS IN ALOPECIA AREATA PATIENTS

[1]Nihan BOZKURT, *[1]Kubra SAHIN, [2]Saime SEZER SONDAS, [1]OMER ATES, [3]Goknur KALKAN

[1]Department of Medical Biology, Medical Faculty, Tokat Gaziosmanpasa University, Türkiye

[2]Malatya Turgut Ozal University, Faculty of Medicine, Department of Medical Biology, Türkiye

[3]Yıldırım Beyazıt University, Faculty of Medicine, Department of Dermatology, Türkiye

Research Article

Received: 31/07/2023, Accepted: 20/08/2023

*Corresponding author: kubra944@hotmail.fr

Abstract

Alopecia areata (AA) is a common, has organ-specific symptoms and an autoimmune disease that targets hair follicles and causes scarring, hair or hair loss. Although the etiopathogenesis of AA has not been clarified yet, it is thought to be a polygenic disease in which autoimmunity and inflammation are effective in addition to psychological factors and heredity. Immunological hair follicle dysfunction process in AA are controlled by activated T cells. It is thought that cytokines released in T cells may cause hair loss in AA. Studies have reported that various genes of the immune system, such as interleukin and cytotoxic T lymphocyte-associated antigen 4 (CTLA4), are effective in the development of the cell membrane, and dysfunction of the CTLA-4 antigen may be associated with various diseases. The CTLA-4 gene polymorphism is thought to be associated with impaired control of T cell proliferation, and this gene is thought to be a candidate gene for susceptibility to autoimmunity. This study was conducted to investigate whether CTLA4 CT60 A/G and +49AG polymorphisms have a role in susceptibility to Alopecia Areata. 195 patients and 173 healthy volunteer controls were included in the study, and genotype and allele frequencies were determined by PCR-RFLP method. The obtained data were analyzed with OpenEpi. Our results showed that the +49AG polymorphism was not associated with AA

susceptibility in the Turkish population, but the CT60 polymorphism might be associated with AA susceptibility. However, more studies are needed to confirm our results.

Key Words: Alopecia areata, CTLA4, Immunity, PCR-RFLP

Özet

Alopesi areata (AA), saç foliküllerini hedef alan ve yara izi, saç veya saç dökülmesine neden olan yaygın, otoimmün bir hastalıktır. AA'nın etiyopatogenezi henüz netlik kazanmamış olmakla birlikte psikolojik faktörler ve kalıtımın yanı sıra otoimmünite ve inflamasyonun da etkili olduğu poligenik bir hastalık olduğu düşünülmektedir. AA'daki kıl folikülü disfonksiyon mekanizmaları immünolojiktir ve aktifleştirilmiş T hücreleri tarafından kontrol edilir. T hücrelerinde salınan sitokinlerin AA'da saç dökülmesine neden olabileceği düşünülmektedir. Çalışmalar, interlökin ve sitotoksik T lenfosit ilişkili antijen 4 (CTLA4) gibi bağışıklık sisteminin çeşitli genlerinin hücre zarının gelişiminde etkili olduğunu ve CTLA-4 antijeninin işlev bozukluğunun çeşitli hastalıklarla ilişkili olabileceğini bildirmiştir. CTLA-4 gen polimorfizminin, T hücre proliferasyonunun bozulmuş kontrolü ile ilişkili olduğu ve bu genin otoimmüniteye yatkınlık için aday bir gen olabileceği düşünülmektedir. Bu çalışma, CTLA4 +49AG ve CT60 polimorfizmlerinin Alopesi Areata'ya duyarlılıkta rolü olup olmadığını araştırmak amacıyla yapılmıştır. 195 hasta ve 173 sağlıklı kontrol çalışmaya dahil edildi ve genotip ve alel frekansları PCR-RFLP yöntemi ile belirlendi. Elde edilen veriler OpenEpi ile analiz edildi. Sonuçlarımız, Türk popülasyonunda +49AG polimorfizminin AA duyarlılığı ile ilişkili olmadığını, ancak CT60 polimorfizminin AA duyarlılığı ile ilişkili olabileceğini göstermiştir. Ancak, sonuçlarımızı doğrulamak için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Alopesi areata, CTLA4, Bağışıklık, PCR-RFLP

1. Introduction

Alopecia areata (AA) is an autoimmune and common disease that targets hair follicles, does not leave scars, and causes sharply round, oval, or circumscribed, hair loss on the scalp or any

part of the body (Yazıcı et al., 2006; Alzolibnani, 2011; Rajabi et al., 2019; Rajabi et al. 2022). The frequency of AA, which is alopecia totalis (AT) in which all hair is shed and alopecia universalis (AU) in which all body hair is involved, varies according to ethnic groups, is about 0.1% worldwide. It can be seen in everyone, regardless of race, gender and age groups (Martinez-Mır et al., 2007). Although there is no gender discrimination in terms of the incidence of AA, the disease progresses more severely especially in men (Alzolibnani, 2011). The disease has a sudden onset, its progression is unpredictable, and it can recur throughout life (Martinez-Mır et al., 2007).

Although the etiopathogenesis of AA has not been clarified yet; It is a polygenic disease in which autoimmune and inflammatory-related genes are effective as well as infections, psychological factors and environmental factors (Yazıcı et al., 2006; Subramanya et al., 2010; Santander et al., 2020). Especially with many genetic studies in recent years, it is aimed to identify AA tendency genes with candidate gene approaches and genome-wide analyzes. These studies show that genes (chemokines, T cell regulatory genes, cytokines, etc.) that play a role in immune mechanisms with AA (Rajabi et al., 2022). It has also been supported by studies that various genes of the immune system such as human leukocyte antigen, cytotoxic T lymphocyte-associated antigen 4 (CTLA4), interleukin genes, and autoimmune regulatory genes contribute to the recovery of this disease (Santander et al., 2020).

The immune response plays a role in the hair follicle dysfunction process in AA, and this process is regulated by activated T cells. Although the function of these T cells in the pathogenesis of the disease is not yet fully understood, it is thought that cytokines released in T cells may be important mediators that cause hair loss in AA (Shimizu et al. 2005).

CTLA4 gene (2q34) is a member of the immunoglobin family and plays a role as a negative regulator in the formation of the T-cell response. Many polymorphisms in the CTLA4 gene have been implicated as susceptibility genes in the genesis of many autoimmune diseases (Santander et al., 2020).

CTLA-4 is a member of the immunoglobulin family and encodes a protein that transmits the signal that inhibits T cell activation and cytokine secretion (Diler 2017; Kırkık et al., 2020). The CTLA4 gene is located on chromosome 2 (q33) and contains 4 exons. These regions encode four different functional domains: a cytoplasmic domain, an extracellular domain, a leader sequence, and a transmembrane domain. Numerous SNPs such as +49 adenine/guanine -318 cytosine/thymine (-318C/T), (+49A/G), and +6230G/A (CT60) have been detected in the CTLA4

gene. Experimental studies in recent years have shown that dysfunction of CTLA-4 antigen leads to various diseases and CTLA4 gene polymorphism is associated with impaired control of T cell proliferation. The gene encoding CTLA4 is also thought to be a candidate gene for susceptibility to autoimmunity. Numerous studies in the Turkish population have shown a relationship between the CTLA-4 gene and many autoimmune diseases such as Behcet's disease, Type 1 diabetes, Graves' disease, Celiac, systemic lupus erythematosus (SLE), and thyroid-related orbitopathy (Diler, 2017).

However, there are some discrepancies in the literature in terms of these data in different populations. This study was conducted to investigate whether CTLA4 CT60A/G and +49AG polymorphisms have a role in susceptibility to Alopecia Areata.

2. Material and Methods

2.1. Patients and sample collection

195 patients (mean age ± SD: 36.2 ± 9.4 years) diagnosed with AA and 173 healthy volunteers with no AA history (mean age ± SD: 37.08 ± 11.13), who applied to the Tokat Gaziosmanpasa University Faculty of Medicine Department of Dermatology, were included in this study. This study, which was conducted in accordance with the Declaration of Helsinki, was approved by the Tokat Gaziosmanpasa University Faculty of Medicine Ethics Committee with the number 2012-GOKAEK-004.

2.2. DNA Isolation

In this study, 200 µl of genomic DNA was obtained from the blood of volunteers in the patients with AA and control groups, taken into 5 µl EDTA tubes, using GeneJET gDNA isolation kit (Thermo Scientific™, USA) in line with the manufacturer's recommended guide.

2.3. Genotyping

The polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP) method was used for the genotyping of the samples used in this study, and the PCR product lengths, and primer sequences, and restriction enzymes are shown in Table 1. PCR was performed in a total volume of 25 µL containing 2.5 µL 10× PCR buffer, 1.5 µL $MgCl_2$ (25 mmol/L), 0.3 µL dNTP (25 mmol/L), 0.8 nmole/µL of each primer, 2 µL gDNA and 1 Unıte Taq polymerase

(Fermentas, Shenzhen, China). %3 agarose gel was used for imaging restriction products and genotyping.

Table 1. Polymorphisms, the PCR primers, annealing temperatures, PCR product lengths, and Restriction Enzymes

SNP	PRIMERS	ANNEALING TEMPERATURE	PCR PRODUCT LENGTHS	RESTRICTION ENZYMES
CTLA 4 CT60 A/G (rs3087243)	5'-ATAATGCTTCATGAGTCAGCTT-3' 5' -GAGGTGAAGAACCTGTGTTAAA-3'	56 ^{0}C	178 bp	Nla III
CTLA 4 +49 A/G (rs231775)	5'-CCACGGCTTCCTTTCTCG-3' 5'-GCAGAAGACAGGGATGAA-3'	56 ^{0}C	313 bp	Bbv I

2.4. Statistical analysis

All statistical data were analyzed with OpenEpi Info software version 3.01. Genotyping results of patients with AA and control group were compared with chi-square or Fisher's exact test. Give the fitness quality of genotypic distributions and Arlequin Software ver. 2000 (University of Geneva, Switzerland) was considered statistically significant when *p* values were less than 0.05. Odds ratios (ORs) and 95% confidence intervals (CIs), chi-square or Fisher's exact test were significant. when it was calculated.

3. Results

The results of our study investigating the possible effects of two SNPs of the CTLA4 gene in the pathogenesis of AA are presented in Table 2. According to our results, no significant correlation was found between CTLA-4 49 A/G gene polymorphism and AA in terms of allele and genotype frequency distribution. The frequencies of AA, GA, and GG genotypes related to CTLA-4 49 A/G gene polymorphism were found to be 51%, 43%, 12% in the patients, and 47,4%, 44,4%, and 8,2% in the control group, respectively (p= 0.69). In terms of allele frequencies, it was found 72,3% for the A allele, 27,7% for the G allele in the patients, and 69,5% for the A allele and 30,5% for the G allele in the control group (p=0.21).

Table 2. Genotype and allele frequencies of CTLA-4 gene polymorphisms (+49A/G and CT60) in patient and control groups

	Patiens with AA n=195	**Control Group n=173**	**P (Pc)**	**O.R (CI 95%)**
CTLA-4 49 A/G			0.6915	
AA	99[51%]	82[47,4%]		
GA	84[43%]	77[44,4%]		
GG	12[6%]	14[8,2%]		
			0.210	
Allele frequency	282[72,3%]	238[69,5%]		
	108[27,7%]	104[30,5%]		
A				
G				
CTLA-4 CT60			**0.0000**	
GG	128[66%]	79[46%]		
GA	8[4%]	55[32%]		
AA	59[30%]	39[22%]		
			0.042	**1.31 (0.97 to 1.77)**
Allele frequency	264[68%]	213[62%]		
G	126[32%]	133[38%]		
A				

In addition, a significant correlation was found between CTLA-4 CT60 A/G gene polymorphism and AA in terms of genotype frequency distribution. The frequencies of GG, GA, and AA genotypes related to the CTLA-4 CT60 A/G gene polymorphism were found to be 66%, 4%, and 30% in the patients, and 46%, 32%, and 22% in the control group, respectively (p= 0.000). In terms of allele frequencies, it was found % for the G allele, % for the A allele in the patients, and 62% for the G allele and 38% for the A allele in the control group (p=0.042).

4. Discussion

In this study, the possible connection of CTLA4 49 A/G and CT60 G/A gene polymorphisms, which are thought to have a critical role in the etiology of AA, was investigated for the Turkish population.

AA, an autoimmune disease, is hair loss associated with many gene variants that play a role in the formation of the immune response (Betz et al., 2015). In experimental studies, it was determined that CTLA4 expressed from T-cells (CD8+ and CD4+) is a receptor responsible for

maintaining autoimmunity. It acts as a negative regulator in the regulation of the T cell response (Rowshanravan, Halliday and Sansom, 2018). CTLA-4 polymorphisms have been related with many autoimmune diseases, but studies on immune-related skin diseases have yielded inconsistent results. For example, no relationship was found between psoriasis and vitiligo and CTLA-4 (Salinas-Santander et al., 2020). However, in their study investigating the possible relationship between CTLA4 and many genetic variants in AA, John et al. showed that CTLA-4 (rs231775 and rs3087243) may be a susceptibility gene for AA (John et al. 2011).

In this context, CTLA4 gene variant studies have been performed in different populations based on the relationship between autoimmunity and AA disease. One of the association studies has suggested that CTLA-4 may be an important risk factor for AA in the European population and is also in connection with the severity of the disease (Salinas-Santander et al., 2020). In another meta-analysis study (2858 AA patients and 5444 healthy control), a meaning relationship was found between CTLA-4 rs231726 polymorphism and AA, but no relationship was ascertained between rs231775 and rs3087243 polymorphisms and AA (Zhou et al., 2022).

In this study; while no association was found between the rs231775 variant and AA susceptibility, rs3087243 was associated with the AA. ($p=0.6915$, $p= 0.0000$), respectively. In a study conducted on the Italian population, results supporting our results were obtained. No association was found with the rs231775 variant, but was associated with the rs3087243 variant (Megiorni et al., 2013). On the other hand, in contrast to the Italian and Turkish populations, the rs3087243 gene variant was not found to be associated with the development of AA in the Iranian population (Moravvej et al., 2018). In a study that included 50 patients and 100 healthy controls in the Mexican population, it was shown that CTLA4 variants (rs3087243 and rs231775) were not effective a risk factor for the evolution of AA in the Mexican population (Salinas-Santander et al., 2020).

In the study, no significant relationship was found between rs231775 and AA. This result indicates that the rs231775 variant is not an effective risk factor for the evolution of AA. Our results show that only CTLA-4 rs3087243 (CT60) polymorphism can be an important genetic marker for the evolution of AA in the Turkish population.

5. Conclusion

In conclusion, CTLA4 rs3087243 genetic variant is a possible risk factor in Turkish population, while rs231775 genetic variant is not a risk factor for the development of AA. In addition, more studies are needed for both the heterogeneous results in the literature and for rs3087243 to be accepted as a risk factor for the development of AA in the Turkish population.

Author contributions: Desing: KS, NB, SSS, OA, GK; collection of data: KS, NB, SSS, OA, GK; Genetic analysis and interperetation of data: KS, NB, literature review: KS, NB writing of article: KS, revision of paper: KS, NB,

Funding: This study was encouraged by Tokat Gaziosmanpasa University Scientific Research Projects (Project Number: 2013/16).

Compliance with ethical standards

Conflict of Interest: All authors confirm that no conflict of interest has occurred.

Ethical approval: Required permissions were approved by Tokat Gaziosmanpasa University Ethics Committee of Clinical Research 2012-GOKAEK-004.

Informed consent: Written informed consent was obtained from all of the registered volunteers.

References

Alzolibani, A. A., (2011). Epidemiologic and genetic characteristics of alopecia areata, Acta Dermatoven APA, 20(4), 191-198.

Betz, R.C., Petukhova, L., Ripke, S., Huang, H., Menelaou, A., Redler, S. et al. (2015). Genome-wide meta-analysis in alopecia areata resolves HLA associations and reveals two new susceptibility loci. Nat Commun, 6(1), 5966. https://doi.org/10.1038/ncomms6966

Budak Diler, S., (2017). Mesane Kanserli Hastalarda CTLA-4 Geni +49 A/G Polimorfizminin Araştırılması. Turkiye Klinikleri J Med Sci, 37(1):16-20.

John, K.K.G., Brockschmidt, F.F., Redler, S., Becker, T., Nöthen, M.M., & Betz, R.C. (2011). Genetic Variants in CTLA4 Are Strongly Associated with Alopecia Areata. Journal of Investigative Dermatology, 131(5), 1169-1172. https://doi.org/10.1038/jid.2010.427

Kırkık, D., Kalkanlı Taş, S., Çağıltay, E., Kalkanlı, N., (2020). Otoimmün tiroid hastalıklarında CTLA-4 geninin in silico analizinin değerlendirilmesi. J. Medıcıne Pallıatıve Care; 1(3): 58-6.

Martınez-Mır, A., Zlotogorski, A., Gordon, D., Petukhova, L., Mo, J., Gilliam, T. C., Londono, D., Haynes, C., Ott, J., Hordinsky, M., Nanova, K., Norris, D., Price, V., Duvic, M., Christiano, A. M., (2007). Genomewide Scan for Linkage Reveals Evidence of Several Susceptibility Loci for Alopecia Areata, The American Journal of Human Genetics, 80, 316-328.

Megiorni, F., Mora, B., Maxia, C., Gerardi, M., Pizzuti, A., Rossi, A. (2013). Cytotoxic T-lymphocyte antigen 4 (CTLA4) +49AG and CT60 gene polymorphisms in alopecia areata: a case–control association study in the Italian population. Arch Dermatol Res, 305, 665-670. https://doi.org/10.1007/s00403-013-1348-3

Moravvej, H., Tabatabaei-Panah, P.S., Abgoon, R., Khaksar, L., Sokhandan, M., Tarshaei, S., *et al.(2018).*Genetic variant association of PTPN22, CTLA4, IL2RA, as well as HLA frequencies in susceptibility to alopecia areata. Immunol Invest, 47,666-679. https://doi.org/10.1080/08820139.2018.1480032

Rajabi, F., Amoli, M. M., Robati, R. M., Nasrabadi, M. A., Jabalameli, N., (2019). Macrophage migration inhibitory factor polymorphism (rs755622) in alopecia areata: a possible role in disease prevention. Arch Dermatol Res, 311(8):589-594. https://doi.org/10.1007/s00403-019-01934-9

Rajabi, F., Abdollahimajd, F., Navid Jabalameli, N., Kashani, M. N., Firooz, A., (2022). The Immunogenetics of Alopecia areata. Adv Exp Med Biol, 1367:1959. https://doi.org/10.1007/978-3-030-92616-8_2.

Rowshanravan, B., Halliday, N., & Sansom, D.M. (2018). CTLA-4: a moving target in immunotherapy. Blood, 131(1), 58-67. https://doi.org/10.1182/blood-2017-06-741033

Salinas-Santander, M.A., Cantu-Salinas, C.S., Ocampo-Candiani, J., Suarez-Valencia, V.J., Ramirez-Guerrero, J.G., Sanchez-Dominguez, C.H. (2020). CTLA4 +49AG (rs231775) and CT60 (rs3087243) gene variants are not associated with alopecia areata in a Mexican population from Monterrey Mexico. Anais Brasileiros de Dermatologia, 95(3), 283-288. https://doi.org/10.1016/j.abd.2020.03.001

Seleit, I., Bakry, O. A., Gayed, E.,A., Gawad, A. E. D., (2018). Polymorphism of FAS and FAS Ligand Genes in Alopecia Areata: A Case-control Study in Egyptian Population. Indian J Dermatol, 63(3):220-226. https://doi.org/10.4103/ijd.IJD_286_17

Shımızu, T., Hizawa, N., Honda, A., Zhao, Y., Abe, R., Watanabe, H., Nishihira, J., Nishimura, M., Shimizu, H., (2005). Promoter region polymorphism of macrophage migration inhibitory factor is strong risk factor for young onset of extensive alopecia areata, Genes and Immunity, 6, 285–289.

Subramanya, R. D., Coda, A. B., Sinha, A. A., (2010). Transcriptional profiling in alopecia areata defines immune and cell cycle control related genes within disease-specific signatures, Genomics, 96, 146–153.

Yazıcı, A. C., Başterzi, A., Acar, Ş. T., Üstünsoy, D., İkizoğlu, G., Demirseren, D., Kanık, A., (2006). Alopesi Areata ve Aleksitimi, Türk Psikiyatri Dergisi, 17,101-106.

Zhou, B., Chen, M., Shang, S., & Zhao, J. (2022). Association of CTLA-4 gene polymorphisms and alopecia areata: a systematic review and meta-analysis. Biomarkers, 27(4), 338-348. https://doi.org/10.1080/1354750X.2022.2046855