Turk J Dermatol 2017;11:75-9 • DOI: 10.4274/tdd.3226

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CTLA-4 +49 A/G, -318 C/T, -1661 A/G, CT60 A/G Gene Polymorphisms in Patients with Pemphigus in Turkey

Türkiye'deki Pemfigus Hastalarının CTLA-4 +49 A/G, -318 C/T, -1661 A/G, CT60 A/G Gen Polimorfizmleri

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Submitted/Geliş Tarihi: 28.01.2017
Accepted/Kabul Tarihi: 06.03.2017

This study was presented in the International Pre IID 2013 Satellite Meeting on Autoimmune Bullous Diseases

Abstract

Objective: Pemphigus, expressing a life-threatening blistering disease, result from autoantibodies against the proteins that mediate intercellular adhesion in desmosomes, namely desmoglein 1 and/or 3. The importance of cytotoxic T lymphocyte-associated antigen (CTLA)-4 in negative regulation of T lymphocytes, which take part in this autoimmune reaction, is well known. Gene polymorphisms regarding this molecule affect autoimmunity. We aimed to determine whether CTLA-4 +49 A/G, -318 C/T, -1661 A/G and CT60 A/G gene polymorphisms cause susceptibility to pemphigus in Turkish population.

Methods: We detected genotypes of the single nucleotide polymorphisms for 118 pemphigus patients and 108 healthy individuals with the help of polymerase chain reaction-restriction fragment length polymorphism method.

Results: Distribution of the CTLA-4 +49 A/G, -318 C/T, -1661 A/G and CT60 A/G allele and genotype frequencies did not differ between pemphigus patients and healthy controls (p=0.643, OR=0.931; p=0.847, OR=1.160; p=0.968, OR=0.975; p=0.173, OR=1.303, respectively).

Conclusion: We concluded that these polymorphisms are not associated with pemphigus susceptibility in Turkish population. This is the first study investigating the possible role of the 4 CTLA single nucleotide polymorphism in pemphigus susceptibility simultaneously. The role of CTLA-4 -1661 A/G gene polymorphism in pemphigus was not studied previously.

Keywords: CTLA-4, autoimmunity, gene, polymorphism, pemphigus, Turkey

Öz

Amaç: Yaşamı tehdit eden bir grup bülleşme gösteren hastalığı ifade eden pemfigus, desmozomlardaki hücreler arası yapışmayı sağlayan ve desmoglein 1 ve/veya 3 isimli proteinlere karşı otoantikorlardan kaynaklanır. Sitotoksik T-lenfosit-ilişkili antijen (CTLA)-4'ün, bu otoimmün reaksiyonda rol alan T lenfositlerin negatif regülasyonundaki önemi iyi bilinmektedir. Bu moleküle ilişkin gen polimorfizmleri otoimmüniteyi etkilemektedir. CTLA-4 +49 A/G, -318 C/T, -1661 A/G and CT60 A/G gen polimorfizmlerinin Türk popülasyonunda pemfigus yatkınlığına yol açıp açmadığını belirlemeyi amaçladık.

Yöntemler: Polimeraz zincir reaksiyonu-restriksiyon fragman uzunluk polimorfizmi metodu yardımıyla 118 pemfigus hastası ve 108 sağlıklı kişinin bu tek nükleotid polimorfizmleri için genotiplerini saptadık.

Bulgular: CTLA-4 +49 A/G, -318 C/T, -1661 A/G ve CT60 A/G allel ve genotip dağılımlarının sıklıkları pemfigus hastaları ve sağlıklı kontroller arasında farklı değildi (sırasıyla p=0,643, OR=0,931; p=0,847, OR=1,160; p=0,968, OR=0,975; p=0,173, OR=1,303).

Sonuç: Bu polimorfizmlerin Türk popülasyonunda pemfigus yatkınlığı ile ilişkili olmadığı sonucuna vardık. Bu, söz konusu 4 CTLA tek nükleotid polimorfizminin pemfigus yatkınlığında olası rolünün eşzamanlı araştırıldığı ilk çalışmadır. CTLA-4 -1661 A/G gen polimorfizminin pemfigustaki rolü daha önce çalışılmamıştır.

Anahtar kelimeler: CTLA-4, otoimmünite, gen, polimorfizm, pemfigus, Türkiye

Introduction

Pemphigus constitutes a number of diseases presenting with mucosal and/or cutaneous blisters which can be life-threatening. The frequencies of these rare diseases vary according to geographical region and ethnic groups (1). The blistering is the result of an autoimmune reaction against desmoglein 1 and/or 3 causing loss of adhesion between keratinocytes. This reaction is mediated by T lymphocytes which recognize epitopes of the extracellular domain of desmoglein molecules (2,3).

The cytotoxic T lymphocyte-associated antigen (CTLA)-4 receptor protein is a co-stimulator that counteracts the CD28-mediated T lymphocyte activation, by binding B-7 ligands with a higher affinity than CD28 (4). Ying et al. (5) showed that CTLA-4, when bound to B-7, inhibited T helper 17-mediated autoimmunity. Anjos et al. (6) detected that T allele at -318 C/T gene polymorphism was associated with 30% increased CTLA-4 production in the inactivated T lymphocytes. The study of Måurer et al. (7) indicated that +49 A/G polymorphism affected proliferation of T lymphocytes by changing intracellular distribution of CTLA-4. In addition, CT60 A/G gene polymorphism alters (8) the frequency of regulatory T lymphocytes, on which CTLA-4 is highly expressed (9), which also brings in mind another path to change the functions of immune system. CTLA-4 gene polymorphisms alter susceptibility to autoimmune diseases (10); including autoimmune endocrinopathies (11) and systemic lupus erythematosus (12).

Pavoni et al. (13) did not find an association of CTLA-4 -318 C/T and +49 A/G polymorphisms with endemic pemphigus foliaceus (PF) (fogo selvagem) in Brazilian population. Pincerati et al. (14) also detected no difference in patients with PF with respect to CT60 A/G polymorphism in Brazilian population. Another study also in Brazilian population, however, showed associations of CTLA-4 -1722 T/C, CTLA-4 -318 C/T and CTLA-4 (AT)n microsatellite polymorphisms with PF (15). Fernandez-Mestre et al. (16) reported a moderate increase in the +49 G/G genotype in pemphigus and statistically significant increase in +49 G/G genotype and +49 G allele in pemphigus vulgaris (PV) in Venezuelan population. Narbutt et al. (17) found no difference in the frequency of genotypes and alleles of +49 A/G CTLA-4 gene polymorphism between the patients with PV and PF with respect to controls in Polish population which point to the differences among populations. The association of a CTLA-4 gene polymorphism with pemphigus in Turkish population has not been studied previously.

We aimed to determine whether CTLA-4 +49 A/G, -318 C/T, -1661 A/G, CT60 A/G gene polymorphisms caused susceptibility to pemphigus in Turkish population. To the best of our knowledge, this was the first study concerning CTLA-4 -1661 A/G gene polymorphism in pemphigus in the literature. Furthermore, none of the studies concerning pemphigus in the literature included these 4 CTLA-4 gene polymorphisms in the same population.

Materials and Methods

A total of 118 consecutive unrelated Turkish patients (69 female, 49 male; aged 51.7±13.4 years), who applied to the departments of Dermatology and Venereology of Akdeniz University, Çukurova University and Ondokuz Mayıs University, between January 2006 and January 2013 diagnosed according to direct immune fluorescence method were enrolled in the study, after informed consent. Control group consisted of ethnically matched 108 individuals (64 female, 43 male; aged 40.5±5.4 years) with no known diagnosis of an autoimmune disease, no family history of pemphigus and accepted to take part in the study after informed consent. Participants who refused to give blood samples were excluded. Participants were enrolled in the study in compliance with the principles of the Declaration of Helsinki. The study was approved by the Ethics Committee of Akdeniz University Faculty of Medicine (8112005-221). For genotyping, polymerase chain reaction (PCR)-restriction fragment length polymorphism was employed. Genomic DNA was isolated from the peripheral blood of individuals by MBI Fermantas DNA isolation kit according to the manufacturer's instructions. After checking the products on ethidium bromide stained gels, the PCR products were purified using phenol/chloroform followed by ethanol precipitation. The amplified fragments were digested with BstEII for +49A/G; MseI(TruI) for -318C/T; MseI for -1661A/G; NcoI for CT60A/G, in CTLA-4 polymorphisms.

Statistical Analysis

Comparisons of genotype frequencies between patients with pemphigus and controls were preformed using chi-square test. Chi-square analysis was used to test for deviation of genotype frequencies from Hardy-Weinberg equilibrium.

Results

Distribution of the CTLA-4 +49 A/G, -318 C/T, -1661 A/G and CT60 A/G allele and genotype frequencies did not differ between patients with pemphigus and healthy controls (p=0.643, OR=0.931; p=0.847, OR=1.160; p=0.968, OR=0.975; p=0.173, OR=1.303, respectively).

When statistical evaluation was performed for men and women separately, we did not detect significant difference between patients and controls with respect to the frequencies of allele or genotypes.

The data concerning these allele and genotype distributions were documented in Table 1.

Analysis of the frequencies of the genotypes showed that the patient and the control groups were in Hardy-Weinberg equilibrium at -318 (p ¼ 0.09 and 0.70), -1661 (p ¼ 0.89 and 0.48) respectively.

The linkage disequilibrium (LD) analysis revealed a tight LD between +49 and CT60 sites with D0 score of 0.948.

Discussion

Our results indicated that there was no association between the 4 CTLA-4 gene polymorphisms, namely +49 A/G, -318 C/T, -1661 A/G, CT60 A/G, and susceptibility to pemphigus with

respect to both allele and genotype distributions in Turkish population. As a multicenter study, which included reference hospitals from the southwestern, northern and southern regions of Turkey, we can consider that our results reflected the Turkish population as homogenous as possible. This is the first study concerning the role of CTLA-4 in pemphigus in Turkish population. To the best of our knowledge, this is also the first study concerning the association of pemphigus and these 4 CTLA-4 gene polymorphisms (+49 A/G, -318 C/T, -1661 A/G, CT60 A/G) simultaneously.

Regarding the CTLA-4 gene polymorphisms and the susceptibility to pemphigus, there are conflicting results in the literature. Characteristics of the previous and current studies were summarized in Table 2. In opposition to the study in Venezuelan population (16) where an increase in +49 G/G genotype in pemphigus and statistically significant increase in +49 G/G genotype and +49 G allele in PV were detected, the results of Pavoni et al. (13) and Narbutt et al. (17) were not consistent with association concerning +49 A/G polymorphism in Brazilian patients with PF and Polish patients with PF and PV, respectively. Pavoni et al. (13) also did not find an association with -318 C/T polymorphism in that study. However, Dalla-Costa et al. (15) detected the opposite.

Table 1. *Allele and genotype frequencies of CTLA-4 +49 A/G, -318 C/T, -1661 A/G, CT60 A/G in patients with pemphigus and healthy controls**

CTLA-4 SNPs	Study population	Alleles (%)		Genotypes (%)		
		A	G	A/A	A/G	G/G
CTLA-4 +49 A/G	Patients with pemphigus	147 (70)	63 (30)	50 (51.5)	47 (44.1)	66 (9.5)
	Healthy controls	136 (68)	63 (32)	43 (46.2)	50 (43.5)	7 (10.2)
CTLA-4 -1661 A/G	Patients with pemphigus	197 (84.9)	35 (15.1)	82 (83.6)	33 (29.7)	1 (2.6)
	Healthy controls	182 (85.1)	32 (14.9)	77 (77.3)	28 (27.1)	2 (2.4)
CTLA-4 CT60 A/G	Patients with pemphigus	115 (50.4)	113 (49.6)	25 (29)	65 (57)	24 (28)
	Healthy controls	119 (56.7)	91 (43.3)	33 (33.7)	53 (51.6)	19 (19.7)
		C	T	C/C	C/T	T/T
CTLA-4 318 C/T	Patients with pemphigus	172 (88.7)	22 (11.3)	75 (76.3)	22 (19.5)	0 (1.3)
	Healthy controls	191 (89.3)	23 (10.7)	86 (85.2)	19 (20.5)	2 (1.2)

CTLA: Cytotoxic T lymphocyte-associated antigen, SNPs: Single nucleotide polymorphism, *Distribution of these CTLA-4 allele and genotype frequencies did not differ between patients and controls (p=0.643, OR=0.931; p=0.847, OR=1.160; p=0.968, OR=0.975; p=0.173, OR=1.303, respectively) and between patients with pemphigus vulgaris (n=107), pemphigus foliaceus (n=7), paraneoplastic pemphigus (n=4) and healthy controls.**

Table 2. Characteristics of previous and current studies

Study (Year)	Population (Ethnicity)	Disease	Case number	Control number	Studied SNPs	Findings (p, OR)
Pavoni et al. (13) (2006)	Brazil	PF	118	291	-318 C/T +49 A/G	NS NS
Fernandez-Mestre et al. (16) (2009)	Venezuela	PV	37	98	+49 A/G	G/G (p=0.011, OR=2.85) G allele (p=0.013, OR=1.92)
Dalla-Costa et al. (15) (2010)	Brazil (Euro + Afro-Brazilian)	PF	229	374	-318 C/T	T allele (p=0.018, OR=0.059), T/T (p=0.054, OR=0.12)
	Afro-Brazilian		91	79	-1722 T/C	C/C (p=0.011, OR=2.85)
	(Euro + Afro-Brazilian)		229 229 229	379 379 379	-1677 G/A+49 A/G 6230 G/A	NS NS NS
Narbutt et al. (17) (2010)	Poland	PV, PF	PV: 40, PF: 14	176	+49 A/G	NS
Pincerati et al. (14) (2010)	Brazil	PF	248	367	CT60 (6230G/A)	NS
Current study	Turkey	PV, PF	PV: 111, PF: 7	108	+49 A/G -318 C/T -1661 A/G CT60 A/G (3'UTR)	NS NS NS NS

PV: Pemphigus vulgaris, PF: Pemphigus foliaceus, SNPs: Single nucleotide polymorphism

It is noteworthy that Brazilian patients with PF were enrolled in both studies. CT60 A/G polymorphism was not associated with PF in Brazilian population.

CTLA-4 -1661 A/G gene polymorphism in pemphigus has not been studied previously in the literature. However, this polymorphism has been linked to several disease groups including malignancies, infections and autoimmune conditions. Yan et al. (18) pointed to the importance of this polymorphism concerning susceptibility to cancers such as gastric cancer and breast cancer, especially in Asian populations, in their meta-analysis. Karimi et al. (19) found this polymorphism to have an important role in the pathogenesis of cytomegalovirus infections and rejection after liver transplantations in patients infected with this virus. The association of CTLA-4 -1661 A/G gene polymorphism and several autoimmune diseases was also studied. Yousefipour et al. (20) detected that this polymorphism was associated with susceptibility to multiple sclerosis in Iranian population. Park et al. (21) found this polymorphism to be significantly higher in patients with Behçet's disease compared to healthy controls.

We can not rule out, in terms of CTLA-4, a disease-modifying effect or an effect concerning severity or resistance to therapy according to this study. In fact, this aspect concerning the autoimmune diseases should be studied in more detail in the literature. The danger for the patients with pemphigus can be associated with the disease itself such as malnutrition, or with the side effects of the treatment modalities such as immunosupression. There is need for new management strategies which are both effective and safe, and modalities concerning CTLA-4 might be a logical option in the future (9). The studies about the role of this regulatory molecule may improve our view related with the management of not only pemphigus but also other autoimmune diseases, chronic infections and malignancies.

Study Limitations

As limitations of the study, we suggest that unidentified polymorphisms of CTLA-4 and many additional polymorphisms in the these genomic region will be required to verify the possible effect of the genes' variability on pemphigus pathogenesis.

Conclusion

We conclude that CTLA-4 +49 A/G, -318 C/T, -1661 A/G, CT60 A/G gene polymorphisms do not have a major role in susceptibility to pemphigus.

Acknowledgement

The study was supported by Akdeniz University Scientific Research Projects Unit. The paper was presented as oral presentation at the 'Meeting of the Autoimmune Blistering Diseases Genetics Consortia', The International Pre IID Satellite Symposium on Autoimmune Bullous Diseases. 6-7 May 2013 Lübeck, Germany. Special Thanks: We want to state that it is an honour to thank Prof. Olcay Yeğin, from the department of pediatric immunology in Akdeniz University, from our hearts, who inspired us to do this scientific search in addition to many others and helped so much in immunological laboratory studies.

Ethics

Ethics Committee Approval: Akdeniz University Faculty of Medicine Ethics Committee (B.30.2.AKD.0.01.00.00/ Ethics/365. 08.11.2005), Informed Consent: A consent form was completed by all participants.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: A.A.K., S.U., E.A., Design: A.A.K., E.A., Data Collection or Processing: N.S., S.G., F.A., Analysis or Interpretation: A.B., A.A.K., E.A., Literature Search: N.S., A.B., Writing: A.B., A.A.K., E.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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