

Interleukin-1 receptor antagonist and tumour necrosis factor-alpha gene polymorphisms in Turkish patients with allergic contact dermatitis

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Background: It has been shown that the family of interleukin-1 receptor antagonist (IL-1 RA) and tumour necrosis factor-alpha (TNF α) genes are polymorphic and related to some inflammatory diseases. Allergic contact dermatitis is the classic presentation of delayed-type hypersensitivity responses to exogenous agents. A number of genes playing role in inflammatory response may be associated with allergic contact dermatitis.

Objectives: To investigate whether there is an association between IL-1RA and TNF α gene polymorphisms and allergic contact dermatitis in Turkish patients with allergic contact dermatitis.

Methods: This study was performed by the collaboration of Departments of Dermatology and Medical Genetics, Ege University, Faculty of Medicine. A total of 50 patients (31 females and 19 males) with allergic contact dermatitis, and 100 age- and sex-matched controls (58 females and 42 males) were included in the study. IL-1RA Variable Number of Tandem Repeats (VNTR) polymorphism in intron 2 and TNF α -308G-A polymorphism were genotyped by using polymerase chain reaction and agarose gel electrophoresis.

Results: The frequency of IL-1RA 1/2 (48%) genotype was significantly higher ($P = 0.002$) in patient group than that is found in control group (22%). The frequency of TNF α (TNF G-308A) G/G genotype was significantly higher in patient group (68%) than that is found in control group (31%) ($P = 0.008$).

Conclusions: Our findings suggest that TNF α (G/G) gene polymorphism may play role in susceptibility to allergic contact dermatitis in Turkish patients.

Key words: allergic contact dermatitis; genes; interleukin-1RA; TNF α . © John Wiley & Sons A/S, 2009.

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Allergic contact dermatitis is a delayed-type hypersensitivity reaction to an allergen to which a patient has previously sensitized. Allergic contact dermatitis may be influenced by genes that modulate cutaneous inflammation. It can be suggested that genetic susceptibility and the interaction between genetic and environmental factors may play an important role in the aetiology of the allergic contact dermatitis (1–4).

The pathogenesis of allergic contact dermatitis involves an initial sensitization phase when the patient first comes in contact with the allergen.

The subsequent re-exposure of the skin leads to reaction (1, 5). In the sensitization phase which is regulated by a network of cytokines, contact with allergic material on the skin leads to the secretion of tumour necrosis factor-alpha (TNF α), granulocyte/macrophage colony-stimulating factor (GM-CSF) by keratinocytes and other cytokines (6, 7).

Inflammatory mediators such as cytokines and chemokines play an important role in skin inflammation by conducting cutaneous inflammatory and repair process (6). Interleukin-1 receptor antagonist (IL-1RA) and TNF α are cytokines which play

roles in inflammatory response. IL-1 (Interleukin-1) family consists of IL-1 α , IL-1 β , IL-1RA. IL-1 α and IL-1 β are synthesized by monocytes, macrophages, neutrophils, hepatocytes, and tissue macrophages (8, 9).

IL-1RA is a member of IL-1 family and binds IL-1 receptors, but it does not create any intracellular reaction. IL-1RA has two variants: sIL-1RA (soluble IL-1RA) and icIL-1RA (intracellular IL-1RA). sIL-1RA is secreted by monocyte, macrophage, neutrophil, and other cells. On the other hand, icIL-1RA is found in the keratinocyte, monocyte, fibroblast, and other epithelial cells (9–11). Kondo et al. reported that IL-1RA inhibits contact hypersensitivity (12). There have been a number of studies investigating TNF α in allergic situations. Besides these, in Westphal's study, it has been shown that TNF α is up-regulated in response to contact allergens (13).

IL-1RA is encoded by the IL1RA gene located at 2q13. A 86-bp tandem repeat polymorphism (VNTR) has been described in Intron 2. This polymorphism has been associated with increased production of IL-1RA protein, and reduced production of IL-1 α protein by monocytes (9). IL-1RA is the anti-inflammatory cytokine and is up-regulated in response to several stimuli like contact allergens. The balance between pro- and anti-inflammatory cytokines is important for the extent and duration of the inflammatory response. IL-1RA*1 and IL-1RA*2 are the most common alleles and total frequencies of these alleles are more than 95%. IL-1RA*1/1 genotype is the most frequent genotype and the second one is IL-1RA*1/2 genotype (14).

It has not been understood why certain individuals are more likely to develop allergic contact dermatitis than others. The individual differences in contact allergy may be based on endogenous factors like molecular, genetic backgrounds, skin sensitivity, race, age, and sex, and exogenous factors like chemical and physical properties of the allergens (15–17). Individual differences in the metabolism of allergens, inflammation, and xenobiotic metabolism can influence susceptibility to allergens as well. Xenobiotic-metabolizing enzymes including cytochrome P450, sulfotransferase, glucuronyltransferase, glutathione-S-transferases, and *N*-acetyltransferases catalyse the metabolism of substances. Genetic polymorphisms of these enzymes can affect individual susceptibility to allergens (2, 7).

We aimed to investigate the possible role of IL-1RA and TNF α (G/A) cytokine gene polymorphisms in susceptibility to contact allergy designing a case–control study.

Patients and Methods

Study population

Fifty patients (31 females and 19 males) with positive patch test reactions and 100 controls (58 females and 42 males) were included in the study. All patients with allergic contact dermatitis had skin lesions and were patch tested with TRUETM (Thin-Layer Rapid Use Epicutaneous) test (Mekos Laboratoried, Hillerød, Denmark). All of the patients were outpatients and had positive patch test reaction. For each patient, a dermatologist completed a checklist on course of the disease, localizations, patch test results, and occupations. Patients with any chronic inflammatory diseases were excluded. The control group consisted of unrelated healthy individuals without a history of eczema and atopy, and other allergic diseases in her/his personal and family history. Patch test was performed according to the recommendations of the International Contact Dermatitis Group (18). The study was approved by the Ethics Committee of the Medical Faculty of the Ege University, Izmir. A written informed consent was obtained from all participants.

DNA sampling and isolation (cytokine gene polymorphisms)

Genomic DNA was extracted from peripheral blood using standard techniques. Polymerase chain reaction (PCR) based genotyping of IL-1RA VNTR (Variable Number of Tandem Repeats in intron 2) was performed. Briefly, the PCR consisted of denaturing at 94°C for 2 min, then 30 cycles at 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 45 seconds, and a final extension at 72°C for 5 min. The following primers were used; IL-1Ra: 5'-CTC AGC AAC ACT CCT AT-3' and 5'-TCC TGG TCT GCA GGT AA- 3'. PCR products were analysed by electrophoresis on 2.0% agarose gel. The polymorphisms of IL-1RA were designated as IL-1RA*1 (four repeats, 410 bp), IL-1RA*2 (two repeats, 240 bp), IL-1RA*3 (five repeats, 500 bp), IL-1RA*4 (three repeats, 325 bp), and IL-1RA*5 (six repeats, 595 bp).

TNF α -308 (G > A) polymorphism was detected using allelic-specific polymerase chain reaction (ASPCR) technique. In this technique allele-specific amplification was performed using three primers. PU: 5'-TCTCGGTTTCTTCTCCATCG-3', PG: 5'-ATAGGTTTTGAGGGGCATGG-3', and PA: 5'-ATAGGTTTTGAGGGGCATGA-3'. For every DNA sample two PCRs were performed with two set of primers (PU-PA:PU-PG). Primers PG and PA only differ in their 3' terminal nucleotide. Amplification does not occur when the 3' nucleotide does not match template DNA. After ASPCR all PCR

Table 1. TRUE™ test allergens and positive patch test reactions

Allergen	n (%)
Nickel sulfate	27 (54)
Wool alcohols (lanolin)	2 (4)
Neomycin sulfate	3 (6)
Potassium dichromate	4 (8)
Caine mix	1 (2)
Fragrance mix	4 (8)
Colophony (colophonium)	4 (8)
Paraben mix	4 (8)
Balsam of Peru (Myroxylon pereirae)	2 (4)
Ethylenediamine dihydrochloride	1 (2)
Cobalt chloride	1 (2)
p-tert-butylphenol formaldehyde resin	2 (4)
Epoxy resin	1 (2)
Carba mix	2 (4)
Black rubber mix	—
Methylchloroisothiazolinone (and) methylisothiazolinone (MCI/MI)	2 (4)
Quaternium-15	3 (6)
Mercaptobenzothiazole	3 (6)
p-Phenylenediamine	4 (8)
Formaldehyde	—
Mercapto mix	1 (2)
Thimerosal	5 (10)
Thiuram mix	5 (10)
Benzocaine	—

products were visualized by electrophoresis on 2% agarose gel.

Statistical analyses

All genotypes were tested for the Hardy-Weinberg equilibrium. Odds ratios (ORs) and exact 95% confidence intervals (CIs) were calculated to compare genotype frequencies. Differences in genotype frequencies were compared using an exact Chi-square test. Logistic regression was calculated for differences regarding polymorphisms between cases and controls. Statistical Package for the Social Sciences (SPSS) (release 15.0 for Windows; SPSS, Chicago, IL) was used in all calculations. A *P*-value below 0.05 was considered to be statistically significant.

Results

Of the 50 patients, aged between 21 and 51 (32.16 ± 7.05), 31 (62%) were female and 29 (38%) were male. Of the 100 controls, aged between 15 and 65 years (38.22 ± 14.13), 58 (58%) were female and 42 (42%) were male.

All lesions in the patients had clinically relevant positive patch test reactions. Allergens and the number of the patients who gave positive reactions are given in Table 1.

The distributions of genotype frequencies of the cytokine gene polymorphisms in cases with allergic contact dermatitis and controls are shown in Table 2.

In the patient group IL-1RA*1/2 genotype and TNF α G/G genotype were significantly higher than

that were found in the control group according to frequencies (*P* = 0.002 and *P* = 0.008, respectively).

To evaluate the association between TNF α and IL-1RA polymorphisms and the risk of allergic contact dermatitis, OR and 95% CI were calculated. Using genotype A/A as a reference, the risk of allergic contact dermatitis was significantly higher in individuals with the genotype G/G of TNF α -308 polymorphism (OR = 8.22, 95% CI = 1.74–38.89, *P* = 0.008). Using genotype 1/1 as a reference, the risk of allergic contact dermatitis was significantly higher in individuals with the genotype 1/2 of IL-1RA polymorphism [OR = 3.32, 95% CI = 1.56–7.05, *P* = 0.002].

The odds ratio (OR) after combination of two genotypes (1/2 + G/G) is 7.33 (95% CI = 2.66 – 20.83) (*P* = 0.001). There were no statistically significant relationship between any allergen and gene polymorphisms. All the genotypes are in Hardy-Weinberg equilibrium (HWE) in both group. Table 3 shows the distribution of alleles in the patients with allergic contact dermatitis and controls.

Discussion

In this study, we found that IL-1RA*1/2 and TNF α G/G polymorphisms were significantly higher in the patients with allergic contact dermatitis compared to the healthy controls. It can be concluded that the patients who have IL-1RA*1/2 gene polymorphism are prone to less effective antagonism to proinflammatory cytokine IL-1. Our results also suggest that TNF α G/G polymorphism may be more related to the sensitivity to contact allergens and TNF α is the more effective proinflammatory cytokine in allergic contact dermatitis pathogenesis.

Previous studies showed that the proinflammatory cytokines may play a crucial role in the aetiology of contact allergy (6, 7, 13, 19, 20). IL-1 β is specifically up-regulated in the epidermis after challenge with allergens. It has also been reported that neutralization of IL-1 β by administration of its natural antagonist, the IL-1 receptor antagonist (IL-1RA) inhibits cutaneous contact hypersensitivity (13).

As genetic polymorphisms affect production of regulatory cytokines, the pathogenesis of allergic contact sensitivity may be influenced by genetic factors. Westphal et al. found that combined deletion of glutathione S-transferase (GST) T1/M1- were associated with thimerosal sensitization (OR = 3.1) (2).

The distribution of polymorphisms of N-acetyltransferases 1 (NAT1) and NAT2 was investigated in patients sensitized to para-substituted aryl compounds and healthy controls. NAT2 rapid acetylators were found more common in the contact allergy (44%) than in the healthy control group (30%,

Table 2. Genotype frequencies in the patients and controls

Locus	Genotype	Patient n (%)	Control n (%)	P	95% CI, OR
IL-1RA	1 1	22 (44.0)	67 (67.0)		
	1 2	24 (48.0)	22 (22.0)	0.002	1.56–7.05, 3.32
	1 3	1 (2.0)	7 (7.0)	0.448	0.05–3.73, 0.43
	2 2	3 (6.0)	2 (2.0)	0.108	0.71–29.13, 4.56
	2 3	0 (0.0)	2 (2.0)	0.999	0, 0
TNF α -308	A/A	2 (4.0)	15 (15.0)		
	G/A	14 (28.0)	54 (54.0)	0.412	0.39–9.5, 1.94
	G/G	34 (68.0)	31 (31.0)	0.008	1.74–38.89, 8.22
	1/2+G/G	16 (72.7)	6 (27.3)	0.001	2.66–20.83, 7.33

Table 3. Allelic frequencies for TNF- α and IL-1Ra in cases and controls

Polymorphism	Allergic contact dermatitis alleles n (%)	Controls alleles n (%)	P	95% CI, OR
TNF α -308				
G	82 (82)	116 (58)	0.001	1.84–5.90, 3.2
A	18 (18)	84 (42)		
IL-1RA (intron 2)				
IL-1RA*1	69 (69)	163 (81.5)	0.209	0.47–30.65, 3.8
IL-1RA*2	30 (30)	28 (14)	0.037	1.14–81.07, 9.6
IL-1RA*3	1 (1)	9 (4.5)		

OR = 1.9). On the other hand, rapid NAT1 phenotype was not significantly different in patients and controls (43% versus 36%, OR = 1.5) (16).

Associations between TNF α -308 polymorphism and various diseases have been increasingly reported to date. TNF α -308 polymorphism may influence other cutaneous inflammatory reactions such as psoriasis (21) and other diseases like idiopathic pulmonary fibrosis (22), ulcerative colitis (23), and acne (TNF α G/A) (24). It has been reported that the carriage of the TNF α -238 A was associated with psoriasis (OR = 3.21, 95% CI 1.60–6.84) (21). TNF α -308 A allele was found higher in idiopathic pulmonary fibrosis (22) and ulcerative colitis (23) patients than controls. On the other hand, TNF α G allele was significantly higher in our patients with allergic contact dermatitis. It can be concluded that G allele of TNF α -308 polymorphism associated with increased skin reactivity.

There are several studies investigating associations between allergic skin disorders and cytokine gene polymorphisms in the literature. Reich et al. found no association between atopic dermatitis and any of the polymorphism found in TNF α , IL-1 β , IL-1RA, IL-6, IL-10 genes (25). On the other hand, it has been reported that IL-5 and IL-5 receptor alpha polymorphisms were associated with atopic dermatitis in Koreans (2). De Jongh et al. found in their case-control study that genetic polymorphisms of TNF α -308 (G/A and A/A) and IL-1 α -889 influenced the susceptibility to chronic irritant contact dermatitis (6). In another study, the relationship between the TNF α -308 A/A genotype and irritant contact dermatitis has been reported (20). Dai et al.

found that the risk of severe generalized dermatitis decreased in patients with genotype TNF α G/A (OR = 0.398, 95% CI 0.164–0.967) (7).

Westphal et al. investigated polymorphisms in the genes encoding for several cytokins including TNF α (G-238 A, G-308 A), IL-1 β , IL-1RA and IL-6 in patients with allergic contact dermatitis. They reported a possible relationship between the TNF α -308 G/A (OR = 1.54, 95% CI 0.92–2.55) and A/A (OR = 2.36, 95% CI 0.84–6.51) polymorphisms and contact allergy (13). In contrast to this report, we found an association between TNF α G/G (OR = 8.22, 95% CI 1.74–38.89) polymorphisms and allergic contact dermatitis. This might be due to the differences between genetic make up of populations in which the studies were performed. It can also be hypothesized the association is the result of linkage disequilibrium of the polymorphism with other alleles.

According to our results, TNF α G/G polymorphism may be more related to the sensitivity to contact allergens than IL-1RA*1/2 gene polymorphism. It can be concluded that TNF α G/G polymorphism is associated with the development of allergic contact dermatitis in Turkish patients.

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