Associations of TNF α –308G>A, TNF α –238G>A, IL-1 α –889C>T and IL-10 –1082G>A Genetic Polymorphisms with Atopic Diseases: Asthma, Rhinitis and Dermatitis

Željka Babić^a Ivana Sabolić Pipinić^a Veda Marija Varnai^a Sanja Kežić^b
Jelena Macan^a

^aInstitute for Medical Research and Occupational Health, Zagreb, Croatia; ^bInstitute of Occupational Health, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Key Words

Atopy · Cytokines · Environmental factors · Genetic factors · Lifestyle · Skin symptoms

Abstract

Background: Polymorphisms of cytokine genes are an interesting focus for association studies involving atopic diseases due to their role in immune cell communications during inflammation. The aim of this study was to investigate associations of TNF α –308G>A, TNF α –238G>A, IL-1 α –889C>T and IL-10 –1082G>A polymorphisms with atopic diseases with adjustment for confounding lifestyle and environmental factors. **Methods:** This study was performed on 356 Croatian students. The diagnosis of atopic asthma, atopic rhinitis and atopic dermatitis was based on symptoms reported by the modified International Study of Asthma and Allergies in Childhood questionnaire and a positive skin prick test (SPT) to at least one common inhalatory allergen. Genetic polymorphisms were genotyped using the polymerase chain reaction-based technique. The influence of personal (gender, body mass index, parental history of atopic disease), lifestyle (cigarette smoking, pet ownership) and environmental (urban/rural residency, residency in continental/Mediterranean

region) factors reported in the questionnaire was investigated by univariate and multivariate analysis. **Results:** Compared to the control subjects, univariate analysis showed a significant negative association of the TNF α –308G>A polymorphism with atopic asthma, atopic dermatitis, asthma and skin symptoms and positive SPT. These observations were confirmed in a multivariate model only for atopic dermatitis and skin symptoms (atopic dermatitis: OR = 0.27; 95% CI 0.07–1.00; p = 0.050; skin symptoms: OR = 0.29; 95% CI 0.10–0.83; p = 0.021). **Conclusions:** The results indicate a protective role of TNF α –308G>A genetic polymorphisms regarding atopic dermatitis and skin symptoms even after controlling for personal, lifestyle and environmental factors. Further studies are needed to elucidate the molecular patterns of this association in atopic dermatitis and other chronic inflammatory skin disorders.

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Introduction

Polymorphisms of cytokine genes are an interesting focus for association studies involving atopic diseases. Cytokines are a class of signaling molecules that mediate

immune cell communications during inflammation typical for these diseases. The induction of a Th2 response is a hallmark of atopic diseases (i.e. expression of cytokines including IL-4, IL-5, IL-9 and IL-13, which induce class switching to IgE, eosinophil and mast cell proliferation, and airway hyperreactivity). However, it is increasingly evident that additional immune and inflammatory responses contribute to this inflammation [1]. In this study we focused on two general proinflammatory cytokines, tumor necrosis factor (TNF) α and IL-1 α , with a described contribution to acute (i.e. epithelial cell activation, vasodilatation, smooth muscle contractility) and chronic (i.e. promotion of nonspecific inflammation) allergic inflammation processes [1–5], and one anti-inflammatory cytokine, IL-10, with an important role in inhibiting immune cell activation [1–4]. Several epidemiological studies have pointed out the activity of chosen proinflammatory cytokines in IgE-mediated allergic reactions. Elevated levels of TNF α and IL-1 α were found in bronchoalveolar lavage fluid, and TNF α was elevated in nasal lavage fluid after allergen provocation [6, 7]. Allergen exposure was shown to increase the production of TNF α in the epidermis of patients with atopic dermatitis, and an increase in TNF α production was also observed in the blood lymphocytes of patients with acute atopic dermatitis [8, 9]. On the other hand, the IL-10 level in sputum was decreased in asthma patients compared with healthy nonsmokers [10].

The production of these cytokines is influenced by genetic and environmental factors [1], and polymorphisms –308G>A and –238G>A in the gene for TNF α , polymorphism –889G>A in the gene for the IL-1 α and polymorphism –1082C>T in the gene for IL-10 are among other genetic factors suspected to predispose an individual to atopic diseases due to their location in the parts of the genes that regulate gene expression or, in other words, affect the amount of produced cytokines. The effects of the studied polymorphisms on the production of related cytokines have not yet been clarified. Most studies consider the association between TNF α –308 polymorphisms and TNF α levels [11–13]. Some studies have suggested that the AG genotype of TNF α –308 is associated with a high production of cytokine, while the GG genotype is low producing [14]. Genetic polymorphisms of TNF α –308 and IL-1 α –889 were found to influence cytokine expression in the stratum corneum, and consequently susceptibility to chronic dermatitis [15, 16].

The epidemiological research conducted with regard to associations between analyzed polymorphisms and atopic diseases has thus far yielded inconclusive results, mostly investigating the TNF α polymorphism and asth-

ma. Approximately half of the studies, including one genome-wide association study, claim no effect [17–29], while others argue for a significant association, either protective or predisposing [30–40]. Regarding atopic rhinitis and dermatitis, the association of TNF α –308 polymorphisms has rarely been studied [14, 41–43]. Additionally, there are only a few studies regarding other selected polymorphisms and atopic diseases, also with conflicting results [14, 22, 29, 40–47]. Furthermore, confounding effects of lifestyle and environmental factors were rarely evaluated in studies assessing the association between genetic factors and atopic diseases. Components of urban air pollution and cigarette smoke have been linked to a higher occurrence and worsening symptoms of atopic asthma and atopic rhinitis [48–51]. Pet ownership modifies the risk of atopic diseases, whilst a family history of atopic disease increases the risk [52–54]. A detailed adjustment for these personal and environmental factors is rarely attempted in the mentioned studies, and each provides only TNF α –308 polymorphisms and asthma associations [32, 33, 36–38].

Therefore, the aim of this study was to investigate associations of TNF α –308G>A, TNF α –238G>A, IL-1 α –889C>T and IL-10 –1082G>A genetic polymorphisms with the atopic diseases: rhinitis, asthma and dermatitis, with adjustment for important lifestyle and environmental factors.

Materials and Methods

Subjects

Participants of this cross-sectional study were freshmen at the University of Zagreb recruited during 2008 and 2009 at the beginning of a prospective study (Croatian Ministry of Science, Education and Sports, grant No. 022-0222411-2410). They responded to an invitation for participation after an oral presentation of the study's objectives and methods at several faculties of the University of Zagreb. The study was designed in accordance with the Helsinki Declaration and approved by the relevant Ethics Committee. All of the subjects were recruited on a voluntary basis. Each subject was informed about the study protocol and signed a consent form.

The number of responders was 440, and for the purpose of this study 437 participants from whom buccal swabs were collected [55] were recontacted by phone in 2014 and asked to accept via mail a consent form for this study, i.e. consent for the analysis of additional cytokine polymorphisms from their buccal swabs. As many as 241 participants could not be reached because either their mobile phone number had changed or they did not answer. Among the remaining participants, 14 disagreed verbally with any further proceeding in this study and 181 gave permission to be sent an informed consent form to their home address. One hundred and fifteen of the 181 participants (response rate 64%) returned the signed consent form. Those who signed the consent form ($n = 115$)

Table 1. Primers used in the genotyping assay

Poly-morphism site	Primer allele 1	Primer allele 2	Common primer	Allele	
				1	2
TNF α -308	GGAGGCTGAACCCCGTCTCT	GAGGCTGAACCCCGTCCC	GAGGCAATAGGTTTGTAGGGGCAT	A	G
TNF α -238	CCCCATCCTCCCTGCTCT	CCCCATCCTCCCTGCTCC	CCAGAAGACCCCTCGGAAT	A	G
IL-1 α -889	GAAATTCTTTAATAATAGT-AACCAGGCAACAT	AATTCTTTAATAATAGT-AACCAGGCAACAC	GAAGGCATGGATTTTGTAGCCTT-CAAT	T	C
IL-10 -1082	CAACACTACTAAGGCTTC-TTTGGGAA	AACACTACTAAGGCTTC-TTTGGGAG	CTTACTTTCCTCTTACCTATCCCTA-CTT	A	G

together with the unreachable subjects (n = 241) served as subjects for this study (n = 356). We based our decision to include the subjects who could not be reached on the recent guidance for ethical issues in epidemiological studies [56, 57], which suggests that, when previous consent is given for the use of samples in medical research, but limited to a specific study, samples may still be used for similar research without new consent. One woman aged 44 was excluded in order to achieve a narrow age range.

Questionnaire

Each participant filled out a general questionnaire containing questions on age, gender, weight, height, smoking status, urban/rural area of residency, residency in the continental/Mediterranean part of Croatia, pet ownership during childhood (cat, dog, bird and rodent ownership for the first time in early childhood, i.e. before 7 years of age, and also owning any of these pets for the first time during later childhood) and parental history of atopic diseases. Body mass index (BMI) was calculated with the provided data on height and weight, and BMI categories were defined as follows: underweight, BMI <18.5; normal weight, BMI 18.5–24.9; overweight, BMI $\geq$ 25. The subjects also filled out the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [58], which included questions about asthma, rhinitis and dermatitis symptoms.

Skin Prick Test

A standard skin prick test (SPT) [59, 60] was performed in all of the subjects with common inhalatory allergens (Allergopharma, Reinbeck, Germany): grass pollen mixture, birch, hazel and weed (*Ambrosia elatior*, *Artemisia vulgaris*) pollens, house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), molds (*Cladosporium herbarum*, *Alternaria alternata*), cat and dog dander and storage mite (*Lepidoglyphus destructor*). The SPTs included testing with a positive control solution of histamine (10 mg/ml) and negative buffer control solution (Allergopharma), and the skin reaction (weal) was evaluated after 15 min. A mean wheal diameter larger than the negative control by more than 3 mm was considered positive, calculated as $(D + d)/2$, where D is the largest longitudinal diameter and d is its midpoint orthogonal diameter.

Disease Definition

Atopic rhinitis was defined as the report of at least two nasal symptoms (runny nose, itchy nose, blocked nose or sneezing) in the previous year and a positive SPT to at least one tested inhalatory allergen. Atopic asthma was defined as the report of wheezing

and/or dyspnea in the previous year and a positive SPT to at least one tested inhalatory allergen. Atopic respiratory disease was defined as the presence of atopic asthma and/or atopic rhinitis. Atopic dermatitis was defined as the report of at least two dermal symptoms (redness, itching, vesicles and rash) in the previous year and a positive SPT to at least one tested inhalatory allergen.

Having wheezing and/or dyspnea was considered as exhibiting asthma symptoms. Displaying two or more of the four nasal symptoms stated above was considered as having rhinitis symptoms. Showing two or more of the four skin symptoms stated above was considered as having symptoms of dermatitis. Control subjects had negative SPTs and none of the groups of symptoms defined above.

Genetic Analysis

DNA extraction from buccal swab samples was performed by KBioscience Ltd. (Hoddesdon, UK) using the 'Klear Gen' extraction methodology (<http://www.kbioscience.co.uk>). Polymorphisms were genotyped using the KASP SNP genotyping system, a homogeneous fluorescent resonance energy transfer (FRET)-based system, coupled with competitive allele-specific PCR (KBioscience Ltd.) [61, 62]. Blind duplicates and Hardy-Weinberg equilibrium tests were used as quality control tests. Table 1 details the primers used, which were designed for use in the KASP genotyping assay.

Statistical Analysis

The characteristics of the subjects were analyzed using descriptive statistics. Deviation from the Hardy-Weinberg equilibrium in the control group was tested by an exact multinomial test. Associations of carrier status (heterozygotes and homozygotes for the polymorphism grouped together) for the TNF α -308G>A, IL-1 α -889C>T and IL-10 -1082G>A genetic polymorphisms with the presence of atopic diseases, symptoms and SPT status, as well as personal, lifestyle and environmental factors, were analyzed univariately by χ^2 or Fisher's exact test (if the expected cell frequency was <5) and then multivariately by calculating the odds ratio (OR) and 95% confidence intervals (CI) by multiple logistic regression analysis. Only personal, lifestyle and environmental factors found to be significantly associated with atopic diseases, symptoms or SPT status in univariate analysis (specifically, gender, continental/Mediterranean residency, history of atopic disease in parents, cat and rodent pet ownership) were introduced as predictors in the models to obtain a favorable ratio of at least 10 cases per predictor. Given the small number of carriers of the TNF α -238G>A poly-

Table 2. Characteristics of the study participants (n = 356; age 18–29 years, interquartile range 18–19, median 19)

	Overall sample (n = 356)	Atopic asthma (n = 26)	Atopic rhinitis (n = 63)	Atopic respiratory disease (asthma and/ or rhinitis; n = 72)	Atopic dermatitis (n = 37)	Controls (n = 140)
Male	92 (26)	8 (31)	18 (29)	22 (31)	8 (22)	28 (20)
Continental residency	280 (79)	21 (81)	56 (89)*	62 (86)	28 (76)	107 (76)
Mediterranean residency	75 (21)	5 (19)	7 (11)*	10 (14)	9 (24)	33 (24)
Urban residency	264 (74)	22 (85)	47 (75)	55 (76)	28 (76)	104 (74)
Rural residency	91 (26)	4 (15)	16 (25)	17 (24)	9 (24)	36 (26)
Current smokers	133 (37)	9 (35)	21 (33)	26 (36)	14 (38)	45 (32)
BMI categories						
Normal weight	274 (77)	22 (85)	50 (79)	59 (82)	31 (84)	106 (76)
Underweight	45 (13)	4 (15)	9 (14)	9 (13)	5 (14)	17 (12)
Overweight	37 (10)	0 (0)	4 (6)	4 (6)	1 (3)	17 (12)
Owning a cat						
In early childhood	85 (24)	6 (23)	14 (22)	18 (25)	16 (43)*	29 (21)
In late childhood	65 (18)	5 (19)	16 (25)	17 (24)	5 (14)	21 (15)
Owning a dog						
In early childhood	115 (32)	6 (23)	22 (35)	24 (33)	15 (41)	42 (30)
In late childhood	108 (30)	9 (35)	15 (24)	18 (25)	8 (22)	46 (33)
Owning a bird pet						
In early childhood	38 (11)	2 (8)	5 (8)	6 (8)	2 (5)	14 (10)
In late childhood	34 (10)	2 (8)	7 (11)	7 (10)	4 (11)	15 (11)
Owning a rodent pet						
In early childhood	25 (7)	3 (12)	4 (6)	6 (8)	3 (8)	9 (6)
In late childhood	41 (12)	6 (23)*	14 (22)*	14 (19)*	6 (16)	13 (9)
History of atopic disease in parents	95 (27)	7 (27)	28 (45)	31 (44)	16 (43)*	25 (18)

Data are presented as n (%). Asterisks (*) indicate a statistically significant difference between subjects with a disease in comparison to control subjects ($p < 0.05$, tested by χ^2 or Fisher's exact test). For 'early childhood' we considered owning the stated pet for the first time at <7 years of age and for 'late childhood' owning the pet for the first time at ≥ 7 years of age.

morphism (10 subjects, i.e. 3% of the total sample), multivariate analysis including this polymorphism could not be performed. All models were free of multicollinearity (checked by inspection of uncentered variance inflation factors). All analyses were performed with STATA/SE 11.2 for Windows [63]. $p < 0.05$ was defined as significant.

Results

Subjects Characteristics

The characteristics of the study participants are summarized in table 2. The age range was relatively narrow (18–29 years, median 19, interquartile range 18–19). Compared to the control subjects, continental residency was significantly more prevalent in subjects with atopic rhinitis. Owning a rodent pet for the first time in late childhood was significantly more frequent among subjects with atopic asthma, atopic rhinitis and either of the two conditions, while owning a cat in early childhood was

significantly more prevalent among subjects with atopic dermatitis. A history of atopic disease in parents was reported significantly more frequently among subjects with atopic dermatitis.

The reporting of disease symptoms in the previous year through the ISAAC questionnaire was as follows: 49 (14% of the overall study sample) reported having wheezing and/or dyspnea, 100 (28%) reported two or more of the four nasal symptoms, 124 (35%) reported either or both asthma and nasal symptoms, and 63 (18%) reported two or more of the four skin symptoms. These data combined with SPT statuses [145 subjects (41%) had a positive SPT] yielded the following frequencies of atopic diseases in our study sample: 26 participants (7%) had atopic asthma, 63 (18%) had atopic rhinitis and 37 (10%) had atopic dermatitis. However, two or more atopic diseases were present in about a third of the affected participants, as illustrated in figure 1. Atopic respiratory disease (i.e. atopic asthma and/or rhinitis) was present in 72 (20%).

Table 3. Frequencies of polymorphism carriers (heterozygotes and homozygotes for the polymorphism are grouped together) in the overall sample, each of the disease subsamples and control subjects

	Genotype success rate in overall sample, %	Overall sample (n = 356)	Atopic asthma (n = 26)	Atopic rhinitis (n = 63)	Atopic respiratory disease (asthma and/or rhinitis; n = 72)	Atopic dermatitis (n = 37)	Controls (n = 140)
TNFA -308G>A	95	77 (23)	1/24 (4)*	10/57 (18)	10/66 (15)*	3/37 (8)*	38/133 (29)
TNFA -238G>A	92	10 (3)	1/25 (4)	2/59 (3)	3/68 (4)	0/36 (0)	2/127 (2)
IL-10 -1082G>A	95	276 (82)	20/25 (80)	52/58 (90)	58/67 (87)	28/37 (76)	107/132 (81)
IL-1α -889C>T	94	156 (47)	10/24 (42)	21/57 (37)	23/66 (35)	15/37 (41)	62/131 (47)

The data are presented as follows: number of carriers of each polymorphism in each subsample/number of successfully genotyped subjects for the polymorphism in that subsample (%). Asterisks (*) indicate a statistically significant difference in the polymorphism carrier status between the subjects with a disease in comparison to control subjects ($p < 0.05$, tested by χ^2 or Fisher's exact test).

Fig. 1. The circles represent each of the atopic diseases; within are the numbers and percentages from the total study sample (n = 356) of subjects with the corresponding disease or combinations of the diseases. Atopic respiratory disease (i.e. atopic asthma and/or rhinitis) was present in 72 individuals (20%). One or more atopic diseases were present in 86 subjects (24%), whilst 270 (76%) were without any atopic disease. Control subjects (n = 140) had a negative SPT and none of the groups of symptoms.

One or more atopic diseases were present in 86 subjects (24%), and 270 (76%) subjects were without any atopic disease. There were 140 control subjects (without symptoms and a positive SPT). Genotype success rates in the overall sample were 95% for the TNFA -308G>A, 92% for TNFA -238G>A, 94% for IL-1α -889C>T and 95% for IL-10 -1082G>A polymorphism. The frequencies of polymorphism carriers in the overall study sample are pre-

Table 4. Characteristics of the multiple logistic regression models with atopic diseases, symptoms and SPT status as outcome variables and the TNFA -308G>A, IL-1α -889C>T and IL-10 -1082G>A genetic polymorphisms as predictors

Outcome variable	Model characteristics	
	p	pseudo R ²
Atopic asthma	0.027	0.16
Atopic rhinitis	0.000	0.22
Atopic dermatitis	0.001	0.18
Atopic respiratory disease	0.000	0.20
Asthma symptoms	0.114	0.08
Nasal symptoms	0.000	0.14
Dermal symptoms	0.001	0.13
Respiratory symptoms	0.000	0.10
Positive SPT	0.000	0.09

Adjusted for gender, residency in continental/Mediterranean part of Croatia, pet ownership (for cat, rodent) and parent history of any of atopic diseases. Atopic respiratory disease was defined as having either or both atopic rhinitis and atopic asthma.

sented in table 3. The genotype frequencies for all of the polymorphisms in the control group met the Hardy-Weinberg equilibrium (data not shown). There were no significant associations between parental history of atopic diseases and the studied polymorphisms or between continental/Mediterranean residency and the studied polymorphisms (data not shown).

Associations of Genetic Polymorphisms with Atopic Diseases, Symptoms and SPT Status

Univariate analysis showed a significant negative association of the TNFA -308G>A polymorphism with

Table 5. Significant associations from multiple logistic regression models in which the TNF α -308G>A polymorphism was a significant predictor of disease presence, symptoms or SPT status

<i>Atopic dermatitis</i>				<i>Skin symptoms</i>			
predictors	OR	95% CI	p	predictors	OR	95% CI	p
TNF α -308G>A carrier	0.27	0.07–1.00	0.050	TNF α -308G>A carrier	0.29	0.10–0.83	0.021
IL-10 -1082G>A carrier	0.66	0.23–1.86	0.428	IL-10 -1082G>A carrier	0.59	0.25–1.40	0.232
IL-1 α -889G>A carrier	0.72	0.31–1.65	0.435	IL-1 α -889G>A carrier	0.80	0.40–1.60	0.523
Male gender	1.29	0.45–3.77	0.634	Male gender	1.08	0.45–2.60	0.855
Continental vs. Mediterranean residency	1.29	0.50–3.37	0.598	Continental residency	1.36	0.60–3.07	0.461
History of atopic diseases in parents	5.78	2.25–14.84	0.000	History of atopic diseases in parents	3.69	1.66–8.20	0.001
Owning a cat				Owning a cat:			
In early childhood	4.35	1.63–11.58	0.003	In early childhood ¹ (<7 years)	3.13	1.39–7.06	0.006
In late childhood	2.05	0.58–7.26	0.266	In late childhood ¹ (>7 years)	1.76	0.62–4.96	0.285
Owning a rodent pet				Owning a rodent pet:			
In early childhood	0.72	0.13–3.88	0.702	In early childhood (<7 years)	0.84	0.20–3.46	0.806
In late childhood	3.80	1.10–13.17	0.035	In late childhood (>7 years)	3.07	1.07–8.78	0.037

Both models were significant ($p < 0.05$). Italics indicate which associations were significant ($p < 0.05$). For ‘early childhood’ we considered owning the stated pet for the first time at <7 years of age and for ‘late childhood’ owning the pet for the first time at ≥ 7 years of age.

atopic asthma. Carriers of this polymorphism were seven times more prevalent in control subjects than subjects with atopic asthma (29 vs. 4%, Pearson’s $\chi^2 = 6.486$, Fisher’s exact $p = 0.009$). Although no significant association with atopic rhinitis was found, the atopic respiratory disease (asthma and/or rhinitis) was significantly negatively associated with the TNF α -308G>A polymorphism, with about twice as many carriers in the control group as in subjects with the disease (29 vs. 15%, Pearson’s $\chi^2 = 4.340$, $p = 0.037$). The TNF α -308G>A polymorphism was also significantly negatively associated with atopic dermatitis, with about 3.5 more carriers in the control than disease subjects (29 vs. 8%, Pearson’s $\chi^2 = 6.623$, Fisher’s exact $p = 0.009$).

Table 4 presents the characteristics of the multiple logistic regression models with atopic diseases, symptoms and SPT status as outcome variables. Table 5 describes multiple logistic regression models in which the TNF α -308G>A polymorphism was a significant predictor. Multivariate analysis confirmed the results for atopic dermatitis, but not for atopic asthma and atopic respiratory disease. The presence of atopic dermatitis was negatively associated with the TNF α -308G>A polymorphism with borderline significance (OR = 0.27; 95% CI 0.07–1.00; $p = 0.050$) and significantly positively associated with a family history of atopic disease, having a cat in early childhood and a rodent pet in late childhood.

Regarding the symptoms and atopic status, univariate analysis showed that asthma symptoms were significantly less common among carriers of the TNF α -308G>A

polymorphism [6 subjects with wheezing and/or dyspnea (13%) vs. 38 controls (29%) were carriers of this polymorphism; Pearson’s $\chi^2 = 4.697$, $p = 0.030$]. Skin symptoms were also significantly less common among carriers of the TNF α -308G>A polymorphism [7 (12%) subjects with two or more skin symptoms vs. 38 (29%) controls were carriers; Pearson’s $\chi^2 = 6.357$, $p = 0.012$]. As for atopic asthma, multivariate analysis did not confirm the univariate association for asthma symptoms. However, multivariate analysis confirmed the result for skin symptoms (tables 4, 5): skin symptoms were negatively associated with the TNF α -308G>A polymorphism (OR = 0.29; 95% CI 0.10–0.83; $p = 0.021$) and positively associated with a family history of atopic diseases, having a cat in early childhood and rodent pet in late childhood.

Carriers of the TNF α -308G>A polymorphism were also significantly less common among subjects with a positive SPT, taken as a general marker of atopy [24 subjects with a positive SPT (17%) vs. 38 control subjects (29%) were carriers of this polymorphism; Pearson’s $\chi^2 = 4.936$, $p = 0.026$], but the multivariate analysis did not confirm this result. Regarding the TNF α -238G>A polymorphism, no significant associations with atopic disease, symptoms or SPT status was found in the univariate analysis. Given the small number of carriers for this polymorphism (10 subjects, 3%) multivariate analysis could not be performed. There were also no significant associations of atopic diseases, symptoms or SPT status with the IL-1 α -889C>T and IL-10 -1082G>A polymorphisms in univariate or multivariate analysis.

Table 6. Comparison of percentages of carriers of the polymorphisms in our study sample versus literature data from European studies

	Asthma, %		Atopic rhinitis, %		Atopic dermatitis, %		Controls, %	
	our findings	literature findings [ref.]	our findings	literature findings [ref.]	our findings	literature findings [ref.]	our findings	literature findings [ref.]
TNF α -308G>A	4	33 [20] to 48 [21]	33	17 [41]	8	23 [43] to 39 [68]	29	23 [43] to 37 [21]
TNF α -238G>A	4	37 [22]	3	9 [41]	0	3 [43] to 9 [42]	2	2 [41] to 14 [77]
IL-10 -1082G>A	80	80 [44] to 85 [22]	90	no studies	76	76 [42] to 95 [43]	81	76 [46] to 94 [22]
IL-1 α -889C>T	43	23 [22]	37	no studies	41	64 [43]	47	31 [22, 43] to 58 [78]

Discussion

We hypothesized that genetic polymorphisms TNF α -308G>A, TNF α -238G>A, IL-1 α -889C>T and IL-10 -1082G>A influence the odds of having atopic diseases and corresponding symptoms. In this study we noted that after adjusting for lifestyle and environmental factors only a negative association between TNF α -308G>A and a skin atopic disorder remained significant.

The frequency of carriers of the TNF α -308G>A polymorphism in our study sample was lower in patients with atopic asthma and patients with atopic dermatitis, and higher in those with atopic rhinitis than that reported in European studies, but was in line with European studies regarding control subjects (table 6). Our most consistent results indicate the protective role of this polymorphism in the occurrence of atopic dermatitis, where a negative association was noted both in univariate and in multivariate analysis after including lifestyle and environmental factors. Regarding chronic skin disorders, the TNF α -308G/A polymorphism was found to be a protective factor for psoriasis [64], and its association with vitiligo was not confirmed [65]. Irritative effects on the skin seem to be facilitated with this type of TNF α polymorphism [66], as well as the development of contact sensitization to chemicals [67, 68]. We found three studies on Caucasians that focus on the association between the TNF α -308 polymorphism and atopic dermatitis. Two of them [42, 43] found no effect, while the third, by Behniafard et al. [14], reported a protective effect of the TNF α -308G>A polymorphism, similar to our study. It was documented that the spontaneous, unstimulated production of TNF α in skin is defective in patients with atopic dermatitis [69–72]. This can be at least partly attributed to the TNF α -308GG genotype (i.e. wild type) which was suggested to be associated with a low production of TNF α [14]. Therefore, it can be speculated that in atopic dermatitis a part of the pathogenesis is related to defective TNF α production and activity in the skin, which is promoted with

the TNF α -308GG genotype. Additionally, the TNF α -308G>A polymorphism was found to be positively associated with serum TNF α levels, but negatively with nasal TNF α levels [34, 41]. It will be of interest to further study the association of TNF α -308 genotypes with TNF α levels in the skin, respiratory mucosa and blood of atopic and nonatopic subjects, keeping in mind the already revealed discrepancy in the influence of TNF α polymorphisms on serum TNF α levels and local TNF α levels in nasal mucosa, as well as generally diminished TNF α levels in the immune cells of patients with atopic dermatitis [69–72]. It should also be noted that in this study the negative association of the TNF α -308G>A polymorphism was stronger with skin symptoms alone, and was not confirmed in multivariate analysis separately for the atopy marker (positive SPT), suggesting an implication in inflammation processes in skin not strictly related to the IgE-mediated pattern.

Our result of a protective effect of the TNF α -308G>A polymorphism regarding atopic asthma (which was however seen only without including lifestyle and environmental factors) is in accordance only with a smaller number of conducted studies [30, 31]. The small number of asthma patients in our study ($n = 26$) limits further confident interpretation.

The frequency of carriers of the TNF α -238G>A polymorphism in our study sample was generally lower than those reported in European studies (table 6). No significant associations were observed with either atopic disease or its component. However, there were very few carriers of this polymorphism (10 subjects), which limits the interpretation of the significance of this polymorphism in our study sample. Previous studies on the Caucasian population found that the TNF α -238G>A polymorphism was associated with an increased risk for asthma [22, 40] and allergic rhinitis [41], while no significant association was found with atopic dermatitis [14, 42, 43].

The frequency of carriers of the IL-10 -1082G>A polymorphism in our study sample was in line with those re-

ported in European studies (table 6), showing that this polymorphism is common in studied European populations, including Croatia. The number of carriers of the IL-1 α -889C>T polymorphism in our study sample was somewhat higher for asthma and somewhat lower for atopic dermatitis than those reported in European studies (table 6). We did not find significant associations of these two polymorphisms with any of the atopic diseases or disease components. Some of the studies in Caucasians found the IL-10 -1082G>A polymorphism not to be significantly associated with asthma [22, 44], while others noted a protective effect on this disease [29] or a susceptible effect [45]. No difference in the prevalence of this polymorphism was found among atopic dermatitis subjects [42, 43, 46, 47]. The IL-1 α -889C>T polymorphism has not been significantly associated with asthma [22, 40] and for atopic dermatitis there are studies demonstrating both no effect [14] and a susceptible effect [43].

Besides associations of atopic diseases with genetic factors, we also observed significant associations of atopic diseases with environmental and lifestyle factors. The co-occurrence of atopic diseases is fairly common [73], and among our subjects with atopic diseases about a third had two or three atopic diseases. A family history of atopy is one of the most consistently implicated predisposing

risk factors for atopic diseases [52, 74], and it was a predisposing factor for atopic dermatitis in our study as well. Our findings of a predisposing effect of a rodent pet on the occurrence of atopic asthma and atopic rhinitis is in line with previous studies [75]. We found a predisposing effect of owning a cat in early childhood on the occurrence of atopic dermatitis, but no clear association with cat exposure in early childhood and eczema development was found in previous studies [53, 76].

In conclusion, the most consistent result indicates the protective role of the TNF α -308G>A polymorphism regarding atopic dermatitis and skin symptoms even after controlling for personal characteristics, lifestyle factors, environmental factors and a history of atopic disease in parents. Further studies are needed to elucidate the molecular patterns of this association in atopic dermatitis and other chronic inflammatory skin disorders.

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