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Željka BABIĆ[1]
Sanja KEŽIĆ[2]
Jelena MACAN[1]

[1] Institute for Medical Research and Occupational Health, Occupational Health and Environmental Medicine Unit, Ksaverska cesta 2,10 001 Zagreb, Croatia
[2] The Academic Medical Center of University of Amsterdam, Coronel Institute of Occupational Health, Meibergdreef 15, 1100 AZ Amsterdam, The Netherlands

Reprints: Ž. Babić <zbabic@imi.hr>

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Individual susceptibility to contact sensitization: the role of TNFα 308G>A polymorphism and atopy

Background: The significance of individual risk factors in the development of contact allergy, such as genetic variation in cytokine genes and atopy, is still not clearly defined. *Objectives:* The aim of this study was to investigate the association between TNFα 308G>A polymorphism or atopy and contact sensitization (CS) in a cohort from the general Croatian population. *Materials & Methods:* The study involved 312 first-year students from the University of Zagreb (median age: 19 years). Methods included a questionnaire and skin prick and patch testing for common inhalable and contact allergens, respectively, as well as genotyping for TNFα 308G>A polymorphism based on buccal swabs. *Results:* CS (positive patch test for ≥1 contact allergen) was reported in 32%, atopy (positive prick test for ≥1 inhalable allergen) in 38%, and TNFα 308G>A polymorphism in 23% of subjects. Based on multivariate analysis, atopy was confirmed as a predictor of CS, poly-sensitization, CS to p-phenylenediamine and cobalt chloride, and self-reported skin symptoms. TNFα 308G>A polymorphism was confirmed as a predictor of CS to p-phenylenediamine (OR: 5.72; 95% CI 1.20-27.28). *Conclusion:* These findings could be relevant for evaluation of individual susceptibility to developing a contact allergy, particularly among persons occupationally exposed to skin hazards.

Key words: asymptomatic contact sensitization, genetic predisposition, patch test, p-phenylenediamine, skin prick test

The significance of individual risk factors in the development of contact allergy, such as genetic variation in cytokine genes and atopy, is still not clearly defined.

In vitro and animal studies indicate that tumour necrosis factor (TNF) α, a cytokine of the innate non-specific immune response, is also involved in various stages of specific immune responses that are important for the pathogenesis of allergic skin disorders. TNFα is produced and secreted mainly by keratinocytes in the epidermis after hapten-induced activation of the innate immune system [1]. Experiments in mice showed that intradermal TNFα injection induces dendritic cell migration to draining lymph nodes [2], while administration of anti-TNFα to trinitrochlorobenzene-sensitized mice prevented cutaneous response to the application of this antigen [3]. It was also shown in experiments on mice deficient in TNFα receptors that signalling through both types of TNFα receptors (p55TNFR and p75TNFR) is important for the development of contact sensitization, specifically, signalling through p55TNFR receptor influences antigen uptake by dendritic cells, while the p75TNFR receptor is important for the migration of these cells from the epidermis to the draining lymph node [1, 4]. In addition to involvement in the sensitization phase, *in vitro* experiments showed that TNFα release during the elicitation phase promotes the migration of circulating effector T lymphocytes to the skin by influencing adhesion molecule and cytokine expression in keratinocytes and dendritic cells [1]. It was also shown that TNFα released from nickel-specific CD4+ T lymphocytes promotes chemokine expression in keratinocytes which can lead to the accumulation of Th1 lymphocytes in the skin and support chronic inflammation [5].

Due to this involvement of TNFα in the development of contact sensitization, polymorphisms in the TNFα gene that can influence TNFα expression are suspected to consequently influence the development of contact sensitization. One of these polymorphisms is 308G>A, located in the promotor region of the TNFα gene. Its influence on TNFα expression is not clear [1, 6], however, it is suspected to increase the expression of TNFα [7]. Genetic association studies have indicated TNFα 308G>A polymorphism to be a protective factor against psoriasis [8], and, less convincingly, against atopic dermatitis [9, 10]. On the contrary, irritative contact dermatitis appears to be worse when associated with this polymorphism, which may also promote the development of contact sensitization [11].

The impact of atopy on the development and course of contact sensitization is also controversial. There are studies that have not found any relation between atopy and allergic contact dermatitis [11-14], whereas others have pointed to atopy as a predictor of poor prognosis [15, 16], and some

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studies indicated that severely expressed atopic diseases may present protection from the development of contact allergy [17, 18].
The aim of this study was to investigate the association between TNFα 308G>A polymorphism or atopic status and contact sensitization (CS), poly-sensitization, and self-reported skin symptoms in a cohort from the general Croatian population.

Subjects and methods

Subjects

The participants of this cross-sectional study were first-year students at several faculties of the University of Zagreb. They were recruited during 2008 and 2009 at the beginning of a prospective study funded by the Croatian Ministry of Science, Education and Sports (grant No. 022-0222411-2410). The recruitment process has been described in detail in previous publications [9, 19]. In short, subjects voluntarily responded to an invitation to participate following an oral presentation of the study's objectives and methods. After being informed about the study protocol, the participants signed a consent form. The study was designed in accordance with the Helsinki Declaration and approved by the relevant ethics committee.

Methods

Each participant completed both a general questionnaire containing questions on age and sex and the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [20] which includes questions about skin and respiratory symptoms related to atopic diseases. Skin symptoms included redness, itching, vesicles, and rash.
A standard skin prick test (SPT) was performed in all subjects for common inhalable allergens (Allergopharma, Reinbeck, Germany): a grass pollen mixture, birch, pollen from hazel and weed (*Ambrosia elatior, Artemisia vulgaris*), house dust mites (*Dermatophagoides pteronyssinus* and *farinae*), molds (*Cladosporium herbarum, Alternaria alternata*), cat and dog dander, and storage mite *Lepidoglyphus destructor*. The SPT also included testing with a positive control solution of histamine (10 mg/mL) and a negative buffer control solution (Allergopharma). The skin reaction (weal) was evaluated after 15 minutes. A mean wheal diameter larger than that for 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 [21, 22]. Patch testing was performed using a standard method [23] with a European standard series [24] of contact allergens (Brial, Greven, Germany) on Curatest (Lohmann, Neuwied, Germany) plaster strips. After a two-day occlusion, skin reactions were read on the second and third day following application of allergens. Erythema, infiltration, and/or vesicles ('+', '++' or '+++') presented on the third day were considered as a positive reaction, according to the morphological criteria recommended by the International Contact Dermatitis Research Group [25].
DNA extraction from buccal swab samples was performed by KBioscience Ltd. (Hoddesdon, UK) using the "Klear Gen" extraction methodology (http://www.kbioscience.co.uk). 308G>A polymorphism of the cytokine gene TNFα was investigated using the KASP SNP genotyping system; a homogeneous fluorescent resonance energy transfer (FRET)-based system, coupled with competitive allele-specific PCR (KBioscience Ltd.) [19, 26, 27]. Primers used for investigation of cytokine gene polymorphism are described in a previous article [9]. Blind duplicates and Hardy-Weinberg equilibrium tests were used as quality control tests.

Statistical analysis

The characteristics of the subjects were analysed using descriptive statistics. Deviation from the Hardy-Weinberg equilibrium in the control group was tested based on an exact multinomial test. Associations between sex, atopy, carrier status for TNFα 308G>A polymorphism, overall and allergen-specific CS, poly-sensitization, and skin symptoms were analysed univariately by $\chi 2$ test, or Fisher's exact test. Participants who had a heterozygous (GA) and homozygous mutant genotype (AA) were considered carriers of TNFα 308G>A polymorphism, in contrast to participants who had a homozygous wild-type genotype (GG) and were considered non-carriers. The decision to group heterozygotes and homozygotes together was based on a small number of homozygotes in the total sample (only one of 298 successfully genotyped subjects had AA genotype). Significant associations with the TNFα 308G>A polymorphism were then tested by multiple logistic regression analysis, calculating the odds ratio and 95% confidence intervals adjusted for sex and atopic status. In the case that all of the subjects with a certain outcome were women (for example, all subjects sensitized to nickel sulphate), an adjustment was made only for atopic status. The overall significance of the model (model p) was used as the measurement of overall model relevance regarding the selection of predictors; if model p was less than 0.05 then the model as a whole fits significantly better (has significantly higher log-likelihood) than a null model (*i.e.* a model with no predictors). Pseudo R^2 is used as a measure of variation in the outcome variable explained by the selected predictors [28]. Higher pseudo R^2 indicates a model which better predicts the outcome (as measured by increase in log-likelihood compared to the log-likelihood of the null model [McFaddens's pseudo R^2]). All analyses were performed using STATA/SE 11.2 for Windows [29], and $p<0.05$ was considered significant.

Disease and control group definitions

Atopy was defined as positive SPT to at least one tested inhalable allergen, and CS as positive patch testing to at least one tested contact allergen. Specific CS was defined as a positive patch test reaction to a specific contact allergen (for example, CS to nickel sulphate). Poly-sensitization was defined as positive patch testing to at least two tested contact allergens. Positive skin symptoms were defined, according to the ISSAC questionnaire, as at least two dermal symptoms (redness, itching, vesicles and rash) in the previous year. The control group was defined as a group of subjects with negative patch testing to all of the tested allergens and without any of the skin symptoms in question during the

Table 1. Characteristics of the study participants (n = 312; age range: 18-29 years; median: 19 years).

	Total *n*=312 (100%)	CS *n*=99 (32%)	Skin symptoms *n*=56 (18%)	Control group *n*=144 (46%)
Male	62/312 (20)	**6/99 (6)***	11/56 (20)	**35/144 (24)**
Positive SPT	120/312 (38)	**41/99 (41)***	**34/56 (61)***	**41/144 (28)**
TNFα 308G>A carrier	69/298 (23)	23/94 (24)	**7/54 (13)***	**38/138 (28)**

*Data are presented as n (%). *a statistically significant difference relative to the control group; p < 0.05 (χ2 or Fisher's exact test). Skin symptoms were defined as at least two dermal symptoms (redness, itching, vesicles or rash) during the previous year. CS: contact sensitization was defined as a positive patch test reaction to at least one of the tested contact allergens. The control group was defined as a group of subjects with negative patch testing to all of the tested allergens and an absence of skin symptoms during the previous year. SPT: skin prick test. Carriers of TNFα 308G>A polymorphism were categorized into a subsample of successfully genotyped subjects. TNFα 308G>A carrier: a participant with a heterozygous (GA) or homozygous mutant genotype (AA) for TNFα 308G>A polymorphism.*

previous year (atopy was not an exclusion criterion for the control group).

Results

Table 1 shows the general characteristics of the study sample (*n*=312; age range: 18-29 years; median: 19 years; interquartile range: 18-19 years). Overall, sensitization to at least one contact allergen was observed in 99 subjects (32%), poly-sensitization in 27 (9%), while 56 subjects (18%) reported skin symptoms. Genotype success rate for TNFα 308G>A polymorphism in the overall sample was 95%. The genotype frequency for the polymorphism in the control group met the Hardy-Weinberg equilibrium (data not shown). In the whole sample (*n*=312), 13% of subjects had positive patch testing to nickel sulphate, 12% to cobalt chloride, 4% to fragrance mix, 3% to *Balsamum peruvianum* and p-phenylendiamine (PPD), 2% to potassium dichromate, and 1% to lanolin alcohol and colophony. Positive patch test reactions to parabens, benzocaine, rubber antioxidants, epoxy resins, quaternium-15, and chlorquinaldol were rare (<1% of subjects), while reactions to thiuram mix, neomycin sulphate, mercapto mix, 4-p-butylphenol-formaldehyde resin, formaldehyde, 2-mercaptobenzothiazole, methylchloroisothiazolinone/methylisothiazolinone, and ethylenediamine dihydrochloride were not noted.

We found a positive association between female sex and CS (*table 1*) ($p<0.001$ [Pearson's χ^2]), as well as between female sex and specific CS to nickel sulphate and cobalt chloride relative to the control group (100% *vs.* 76%; $p<0.001$ [Fisher's exact test], and 97% *vs*. 76%; $p=0.002$ [Fisher exact test], respectively). Multivariate analysis for sex, atopic status, and TNFα 308G>A carrier status as predictors confirmed significant associations between sex and CS or specific CS to cobalt chloride (*table 2*). Female sex was also significantly positively associated with poly-sensitization (100% *vs*. 76%; $p=0.002$ [Fisher's exact test]). There was no significant association between sex and skin symptoms. Atopy was more frequent among subjects with CS and subjects with skin symptoms than among control subjects ($p=0.036$ and $p<0.001$ [Pearson's χ^2], respectively) (*table 1*). We also noted positive associations between atopy and specific CS to para-phenylenediamine (PPD) or cobalt chloride relative to the control group with borderline significance for PPD and clear significance for cobalt chloride (5/8 [63%] subjects with sensitization to PPD *vs*. 41/144 [28%] control subjects had positive SPT; $p=0.055$ [Fisher's exact test], and 18/38 [47%] subjects with sensitization to cobalt chloride vs. 41/144 [28%] control subjects had positive SPT; $p=0.027$ [Pearson's χ^2]). Atopy was also significantly positively associated with poly-sensitization (15/27 [56%] poly-sensitized subjects *vs.* 41/144 [28%] control subjects had positive SPT; $p=0.006$ [Pearson's χ^2]). Multivariate analysis confirmed these results (*table 2*). There were no other significant associations between sex or atopic status and specific CS.

Association between TNFα 308G>A polymorphism and overall CS or poly-sensitization was not found. This polymorphism was less frequent in atopic than non-atopic subjects (19/116 [16%] vs. 50/182 [27%], respectively; $p=0.033$ [Pearson's χ^2]), and in subjects with skin symptoms relative to control subjects (*table 1*) ($p=0.032$ [Pearson's χ^2]). In contrast, the TNFα 308G>A polymorphism was positively associated with CS to PPD. Five of eight subjects (63%) with CS to PPD, and 38 of 138 control subjects (28%) were TNFα 308G>A carriers ($p=0.04$ [Fisher's exact test]). Characteristics of the participants with sensitization to PPD are shown in *table 3*. It is clear that half of them were poly-sensitized, and only one subject reported skin symptoms in a previous year. Based on the multivariate analysis, TNFα 308G>A polymorphism was confirmed as a significant predictor of CS to PPD but a significant association with skin symptoms was not confirmed (*table 2*). There were no other significant associations between TNFα 308G>A polymorphism and specific CS.

Discussion

This study examined the relationship between CS, atopy, and TNFα 308G>A polymorphism in a cohort of young adults from the general Croatian population. The main findings were a positive association between atopy and CS, poly-sensitization or skin symptoms, and between atopy or TNFα 308G>A polymorphism and CS to PPD.

The role of atopy in the development of CS is not yet clear, with epidemiological studies indicating both significant positive [15, 16] and negative [17, 18] associations, while others have found no significant correlation [11-14, 30, 31]. A recent meta-analysis also showed results that were not entirely consistent; no significant association was demonstrated between atopic dermatitis and contact

Table 2. Multivariate logistic regression analysis for contact sensitization and skin symptoms showing predictors of outcome (sex, atopy or TNFα 308G>A).

	CS		Poly-sensitization		Skin symptoms		CS to PPD		CS to cobalt chloride	
Predictors	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Sex (F/M)	5.99 (2.34-15.32)	**<0.001***	-	-	1.72 (0.76-3.91)	0.197	0.30 (0.03-2.77)	0.290	15.80 (2.04-122.43)	**0.008***
Atopy	2.31 (1.28-4.18)	**0.006***	3.69 (1.53-8.86)	**0.004***	4.25 (2.14-8.44)	**<0.001***	6.48 (1.34-31.39)	**0.020***	3.08 (1.40-6.80)	**0.005***
TNFα 308G>A carrier	0.97 (0.52-1.83)	0.933	1.66 (0.66-4.22)	0.283	0.50 (0.20-1.25)	0.138	5.72 (1.20-27.28)	**0.029***	0.97 (0.40-2.36)	0.948
Model P	<0.001		0.01		<0.001		0.016		<0.001	
Pseudo R^2	0.075		0.064		0.106		0.167		0.113	

*Results are presented as odds ratio (OR) with 95% confidence interval (95% CI). *Significant predictors in the models. CS: contact sensitization was defined as positive patch testing to at least one tested contact allergen; Poly-sensitization: positive patch testing to at least two tested contact allergens; Skin symptoms: at least two dermal symptoms (redness, itching, vesicles or rash) during the previous year; CS to PPD: contact sensitization to p-phenylenediamine; F: female; M: male; Atopy: positive SPT to at least one inhalable allergen; TNFα 308G>A carrier: a participant with a heterozygous (GA) or homozygous mutant genotype (AA) for TNFα 308G>A polymorphism. In a model with poly-sensitization as a dependent variable, gender was not analysed as a predictor because all poly-sensitized subjects were female; Model p: the overall significance of the model was used as the measurement of overall model relevance regarding the selection of predictors, if model p was less than 0.05 then the model as a whole fits significantly better (has significantly higher log-likelihood) than a null model (i.e. a model with no predictors). Pseudo R^2: McFaddens's pseudo R^2 as a measure of variation in the outcome variable based on selected predictors; higher pseudo R^2 indicates a model which predicts the outcome more effectively (as measured by increase in log-likelihood compared to the log-likelihood of the null model).*

sensitization, however, contact sensitization is increased in individuals with atopic dermatitis in general population studies [32]. This study supports atopy as an independent risk factor for the development of contact allergy, defined by positive patch testing to at least one common contact allergen, as well as specific CS to PPD and cobalt chloride. Atopy was also significantly positively associated with poly-sensitization which we defined as positive patch testing to at least two of the tested contact allergens. Any comparison of results between studies is difficult due to the variety of definitions of atopy used among researchers, ranging from self-reported personal history of atopic diseases, clinical history of atopic dermatitis determined by a physician, to objective markers such as positive SPT [14, 32]. It should be pointed out that the objective definition of atopy used in our study, *i.e*. SPT to at least one tested inhalable allergen, may not confirm or exclude predisposition to atopic diseases. The possible reasons for the presented significant associations between atopy and contact sensitization could lie in an impaired skin barrier function in atopic individuals, even with skin visibly unaffected by disease, since it was shown that uninvolved skin of patients with atopic dermatitis is significantly more permeable to chemicals than the skin of non-atopic individuals [33, 34].

We are also aware that in the recent literature, multiple contact allergies are defined as three or more contact allergies in the same person [35-37]. However, in our study, very few participants were poly-sensitized to three or more contact allergens (only four in the entire study sample), therefore we reported results regarding participants sensitized to at least two tested contact allergens (*n*=27). In addition, since frequency of multiple allergies increases with age [35], the results of our study on young adults could be useful for monitoring age-related trends in poly-sensitization in the Croatian population. As an overall limitation, we should also mention that reading was not performed on Day 7 during the patch testing, as suggested in more recent recommendations [38]. Patch testing of the participants was performed during 2008 and 2009, and recommendations for reading skin reactions on the second and third day, that were valid at that time, were followed [23, 24].

The association between TNFα 308G>A polymorphism and the development of contact allergy has previously been indicated, but only for poly-sensitized subjects and for some specific contact allergens, such as chromates, trichloroethylene, and PPD [39-43]. Our study did not confirm an association between contact poly-sensitization and this polymorphism, but supports its association with CS to PPD. This association should be interpreted with reservation due to the small number of subjects with CS to PPD in our study. Atopy and TNFα 308G>A polymorphism were identified as independent risk factors for CS to PPD. Based on a case-control study from Germany, Blomeke *et al*. [42] demonstrated that carriers of TNFα 308G>A polymorphism were significantly more frequent among individuals with a history of allergic contact dermatitis and sensitization to PPD than among age- and sex-matched controls. In another case-control study from Germany, Westphal *et al*. [40] found that contact sensitization to para-substituted aryl compounds (including PPD) and at least one other structurally unrelated allergen was significantly positively associated with TNFα 308G>A polymorphism when compared to control subjects without

Table 3. Characteristics of subjects with contact sensitization to p-phenylenediamine (aged 18-21 years).

Subject	Sex	SPT	Skin symptoms	TNFα 308G>A carrier	Other contact sensitization
1	F	+	-	+	Nickel sulphate
2	M	+	-	-	-
3	F	+	+	-	-
4	F	+	-	+	Parabens
5	F	+	-	+	Nickel sulphate
6	F	-	-	-	-
7	F	-	-	+	-
8	F	-	-	+	Potassium dichromate

F: female; M: male; SPT: positive SPT was defined as a positive SPT reaction to at least one inhalable allergen; Skin symptoms: at least two dermal symptoms (redness, itching, vesicles or rash) during the previous year; TNFα 308G>A carrier: a participant with a heterozygous (GA) or homozygous mutant genotype (AA) for TNFα 308G>A polymorphism; Other contact sensitization: contact sensitization was defined as a positive patch test to the tested contact allergen.

a history of eczema. However, there are some differences between these two studies and our study. Besides TNFα 308G>A polymorphism, other predictive risk factors for CS to PPD investigated by Blomeke *et al.* were sex and age, while in our study these factors were sex and atopy. Due to the narrow age range, age was not considered a predictor in our study. On the other hand, adjusting for atopy adds to the quality of evidence since atopy was significantly positively associated with CS to PPD in our study and judging by the literature, the role of atopy in the development of CS has not yet been clarified. In a study by Westphal *et al*., predictive risk factors for CS to PPD included additional genetic factors (TNFα 238G>A, interleukin [IL]-1β 511C>G, and IL-6 174G>A polymorphisms, as well as several polymorphisms within a variable number of tandem repeats of intron 2 of the IL-1 receptor antagonist gene), but not sex or atopy. There are also differences in the subject selection process between the studies. A novel aspect of our study was that subjects were from a cohort comprising mainly a young general population. The selection process in the study of Blomeke *et al.* was not described in enough detail to exclude the possibility that subjects and controls corresponded to different groups of hospital patients, in which case our selection process would lead to a smaller selection bias [44]. In the study of Westphal *et al*., subjects were recruited from various departments of dermatology in Germany, and the control subjects were healthy individuals recruited from the blood donor registry (a history of eczema was checked using a physician-administered questionnaire).

A small number of subjects sensitized to PPD can be expected in a cohort of young adults from the general population, with hair dyes as the main source of exposure. It should be mentioned that, besides the expected female predominance among subjects sensitized to PPD, almost all of the sensitized subjects (seven out of eight) were asymptomatic, *i.e*. without skin symptoms. Additionally, while 32% of our subjects showed contact sensitization, only 18% reported skin symptoms, and self-reported skin symptoms generally may not be related solely to allergic contact dermatitis. The clinical relevance of asymptomatic contact sensitization has not yet been established. Our recent follow-up study showed that 30% of persons with asymptomatic contact sensitization developed eczema after several years, but also demonstrated that 90% of the positive patch test reactions performed during the first visit were no longer performed during control patch testing [45]. However, it would be interesting to study the outcome of asymptomatic CS to PPD in our young females, keeping in mind that PPD is a common general and occupational contact allergen [46].

In conclusion, this study adds to the scarce pool of evidence regarding the association between TNFα 308G>A polymorphism and sensitization to PPD, with new insight into an association between atopy and CS. The association between atopy and CS, skin symptoms in general, or CS to PPD and cobalt chloride, as well as the association between atopy/TNFα 308G>A polymorphism and CS to PPD deserve further investigation using larger samples. These findings, together with more data on the clinical relevance of asymptomatic contact sensitization, may be relevant in the evaluation of individual susceptibility to developing skin symptoms, particularly among professionals occupationally exposed to skin allergens and irritants, and thus contribute to the prevention of occupational skin diseases [45, 47]. ■

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References

1. Dittmar D, Schuttelaar ML. Immunology and genetics of tumour necrosis factor in allergic contact dermatitis. *Contact Dermatitis* 2017;76:257-71.

2. Cumberbatch M, Kimber I. Dermal tumour necrosis factor-alpha induces dendritic cell migration to draining lymph nodes, and possibly provides one stimulus for Langerhans' cell migration. *Immunology* 1992; 75: 257-63.

3. Piguet PF, Grau GE, Hauser C, Vassalli P. Tumor necrosis factor is a critical mediator in hapten induced irritant and contact hypersensitivity reactions. *J Exp Med* 1991; 173: 673-9.

4. Becke FM, Hehlgans T, Brockhoff G, Männel DN. Development of allergic contact dermatitis requires activation of both tumor necrosis factor-receptors. *Eur Cytokine Netw* 2001; 12: 45-50.

5. Albanesi C, Scarponi C, Sebastiani S, *et al.* A cytokine-to-chemokine axis between T lymphocytes and keratinocytes can favor Th1 cell accumulation in chronic inflammatory skin diseases. *J Leukoc Biol* 2001; 70: 617-23.

6. Mekinian A, Tamouza R, Pavy S, *et al.* Functional study of TNF-α promoter polymorphisms: literature review and meta-analysis. *Eur Cytokine Netw* 2011; 22: 88-102.

7. Louis E, Franchimont D, Piron A, *et al.* Tumour necrosis factor (TNF) gene polymorphism influences TNF-alpha production in lipopolysaccharide (LPS)-stimulated whole blood cell culture in healthy humans. *Clin Exp Immunol* 1998; 113: 401-6.

8. Zhuang L, Ma W, Cai D, Zhong H, Sun Q. Associations between tumor necrosis factor-α polymorphisms and risk of psoriasis: a meta-analysis. *PLoS One* 2013; 8: e68827.

9. Babić Ž, Sabolić Pipinić I, Varnai VM, Kežić S, Macan J. Associations of TNFα -308G>A, TNFα -238G>A, IL-1α -889C>T and IL-10-1082G>A genetic polymorphisms with atopic diseases: asthma, rhinitis and dermatitis. *Int Arch Allergy Immunol* 2016; 169: 231-40.

10. Behniafard N, Gharagozlou M, Farhadi E, *et al.* TNF-α single nucleotide polymorphisms in atopic dermatitis. *Eur Cytokine Netw* 2012; 23: 163-5.

11. Kezic S, Visser MJ, Verberk MM. Individual susceptibility to occupational contact dermatitis. *Ind Health* 2009; 47: 469-78.

12. Li LF, Liu G, Wang J. Prevalence and 1-year outcome of facial allergic contact dermatitis in patients patch tested in a university hospital. *Contact Dermatitis* 2007; 57: 187-90.

13. Wongpiyabovorn J, Puvabanditsin P. Result of standard patch test in patients suspected of having allergic contact dermatitis. *J Med Assoc Thai* 2005; 88: S177-83.

14. Spiewak R. Contact dermatitis in atopic individuals. *Curr Opin Allergy Clin Immunol* 2012; 12: 491-7.

15. Cahill J, Keegel T, Dharmage S, Nugriaty D, Nixon R. Prognosis of contact dermatitis in epoxy resin workers. *Contact Dermatitis* 2005; 52: 147-53.

16. Slodownik D, Wohl Y, Mansura A, *et al.* Allergic contact dermatitis among maintenance and clerical workers in a military population. *Contact Dermatitis* 2006; 55: 335-7.

17. Thyssen JP, Johansen JD, Linneberg A, Menné T, Engkilde K. The association between contact sensitization and atopic disease by linkage of a clinical database and a nationwide patient registry. *Allergy* 2012; 67: 1157-64.

18. Uehara M, Sawai T. A longitudinal study of contact sensitivity in patients with atopic dermatitis. *Arch Dermatol* 1989; 125: 366-8.

19. Sabolić Pipinić I, Varnai VM, Turk R, Breljak D, Kežić S, Macan J. Low frequency of filaggrin null mutations in Croatia and their relation with allergic diseases. *Int J Immunogenet* 2013; 40: 192-8.

20. Asher MI, Keil U, Anderson HR, *et al.* International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995; 8: 483-91.

21. Dreborg S, Frew A. Position paper - allergen standardization and skin tests. *Allergy* 1993; 48: 49-54.

22. Bousquet J, Heinzerling L, Bachert C, *et al.* Practical guide to skin prick tests in allergy to aeroallergens. *Allergy* 2012; 67: 18-24.

23. Wahlberg JE. Patch testing. In: *Textbook of Contact Dermatitis. 1st Ed.* Rycroft RJG, Menné T, Frosch PJ, Benezra C. Berlin: Springer, 1992: 239-68.

24. Bruynzeel DP, Andersen KE, Camarasa JG, Lachapelle JM, Menné T, White IR. European Environmental and Contact Dermatitis Research Group (EECDRG). The European standard series. *Contact Dermatitis* 1995; 33: 145-8.

25. Fregert S. *Manual of Contact Dermatitis. 2nd Ed.* Munksgaard, Copenhagen: Year Book Medical Publishers Inc, 1981: 71-81.

26. Landeck L, Visser M, Kezic S, John SM. Impact of tumour necrosis factor-α polymorphisms on irritant contact dermatitis. *Contact Dermatitis* 2012; 66: 221-7.

27. Landeck L, Visser M, Kezic S, John SM. IL1A -889 C/T gene polymorphism in irritant contact dermatitis. *J Eur Acad Dermatol Venereol* 2013; 27: 1040-3.

28. Mittlböck M, Schemper M. Explained variation for logistic regression. *Stat Med* 1996; 15: 1987-97.

29. StataCorp: Stata Statistical Software: Release 11. College Station, StataCorp LP, 2009.

30. Nielsen NH, Menné T. The relationship between IgE-mediated and cell-mediated hypersensitivities in an unselected Danish population: the Glostrup Allergy Study. *Denmark. Br J Dermatol* 1996; 134: 669-72.

31. Spiewak R. Atopy and contact hypersensitivity: a reassessment of the relationship using objective measures. *Ann Allergy Asthma Immunol* 2005; 95: 61-5.

32. Hamann CR, Hamann D, Egeberg A, Johansen JD, Silverberg J, Thyssen JP. Association between atopic dermatitis and contact sensitization: a systematic review and meta-analysis. *J Am Acad Dermatol* 2017; 77: 70-8.

33. Jakasa I, de Jongh CM, Verberk MM, Bos JD, Kezic S. Percutaneous penetration of sodium lauryl sulphate is increased in uninvolved skin of patients with atopic dermatitis compared with control subjects. *Br J Dermatol* 2006; 155: 104-9.

34. Jakasa I, Verberk MM, Esposito M, Bos JD, Kezic S. Altered penetration of polyethylene glycols into uninvolved skin of atopic dermatitis patients. *J Invest Dermatol* 2007; 127: 129-34.

35. Carlsen BC, Menné T, Johansen JD. 20 Years of standard patch testing in an eczema population with focus on patients with multiple contact allergies. *Contact Dermatitis* 2007; 57: 76-83.

36. Schnuch A, Westphal G, Mössner R, Uter W, Reich K. Genetic factors in contact allergy-review and future goals. *Contact Dermatitis* 2011; 64: 2-23.

37. Friedmann PS, Sanchez-Elsner T, Schnuch A. Genetic factors in susceptibility to contact sensitivity. *Contact Dermatitis* 2015; 72: 263-74.

38. Johansen JD, Aalto-Korte K, Agner T, *et al.* European Society of Contact Dermatitis guideline for diagnostic patch testing - recommendations on best practice. *Contact Dermatitis* 2015; 73: 195-221.

39. Colagiovanni A, Di Renzo L, Sarlo F, Schiavino D, De Lorenzo A. Role of TNF-alpha polymorphism in patients with nickel allergy: a marker of susceptibility to contact polysensitization. *Eur Rev Med Pharmacol Sci* 2016; 20: 2663-6.

40. Westphal GA, Schnuch A, Moessner R, *et al.* Cytokine gene polymorphisms in allergic contact dermatitis. *Contact Dermatitis* 2003; 48: 93-8.

41. Wang BJ, Shiao JS, Chen CJ, Lee YC, Guo YL. Tumour necrotizing factor-alpha promoter and GST-T1 genotype predict skin allergy to chromate in cement workers in Taiwan. *Contact Dermatitis* 2007; 57: 309-15.

42. Blömeke B, Brans R, Dickel H, *et al.* Association between TNFA-308 G/A polymorphism and sensitization to para-phenylenediamine: a case-control study. *Allergy* 2009; 64: 279-83.

43. Dai Y, Leng S, Li L, *et al.* Genetic polymorphisms of cytokine genes and risk for trichloroethylene-induced severe generalized dermatitis: a case-control study. *Biomarkers* 2004; 9: 470-8.

44. Lambert J. Statistics in brief: how to assess bias in clinical studies? *Clin Orthop Relat Res* 2011; 469: 1794-6.

45. Macan J, Rimac D, Kežić S, Varnai VM. Occupational and non-occupational allergic contact dermatitis: a follow-up study. *Dermatology* 2013; 227: 321-9.

46. Thyssen JP, Andersen KE, Bruze M, *et al.* p-Phenylenediamine sensitization is more prevalent in central and southern European patch test centres than in Scandinavian: results from a multicentre study. *Contact Dermatitis* 2009; 60: 314-9.

47. Samardžić T, Varnai VM, Bakotić M, *et al.* Skin health and safety at work in Croatian hairdressing apprentices. *Contact Dermatitis* 2016; 75: 25-31.