

ORIGINAL ARTICLE

Cutaneous delayed-type hypersensitivity in patients with atopic dermatitis

Dana Malajian, BA,^a and Donald V. Belsito, MD^b
New York, New York

Background: Literature on the relationship between atopic dermatitis (AD) and cutaneous delayed-type hypersensitivity is inconclusive.

Objective: We sought to compare the rates of positive patch test reactions to allergens on the North American Contact Dermatitis Group (NACDG) standard tray among patients with and without AD, and, to assess whether atopic patients in our database were more likely to patch test positive to certain classes of allergens.

Methods: A total of 2305 patients underwent patch testing to the NACDG standard screening series. The incidence of positive patch test reactions among patients with AD (n = 297) and without AD (n = 2008) was assessed. Statistical analysis was done using a χ^2 test with Yates continuity correction.

Results: Compared with nonatopic patients, those with AD were statistically more likely to have positive patch tests. AD was associated with contact hypersensitivity to nickel, cobalt, and chromium, but was not associated with contact sensitization to fragrances.

Limitations: Only patients suspected of having allergic contact dermatitis were tested. Our population was geographically limited to metropolitan Kansas City (including Kansas City, MO, Kansas City, KS, and the adjoining suburbs).

Conclusion: Compared with nonatopics, patients with AD are significantly more likely to have at least 1 positive patch test reaction and to develop contact hypersensitivity to metal allergens. (J Am Acad Dermatol 10.1016/j.jaad.2013.03.012.)

Key words: allergy; atopic eczema; chromium; cobalt; contact dermatitis; hypersensitivity; nickel; patch testing.

Atopic dermatitis (AD), also known as atopic eczema, is a chronic inflammatory skin condition whose prevalence is increasing in the general population.¹ It has been reported that up to 30% of children and between 2% and 10% of adults experience AD.² In addition, 50% to 80% of those with AD will go on to develop asthma or allergic rhinitis later in life; these conditions are termed the “atopic triad,” and are believed to be T helper cell type 2 (Th2)-mediated processes.^{3,4}

Cutaneous delayed-type hypersensitivity (CDTH) is a T cell-mediated hypersensitivity response that

Abbreviations used:

AD:	atopic dermatitis
CDTH:	cutaneous delayed-type hypersensitivity
FLG:	filaggrin
NACDG:	North American Contact Dermatitis Group
Th:	T helper cell
TLR:	Toll-like receptor

presents as allergic contact dermatitis with pruritic, eczematous lesions. Nickel is the most common nonplant allergen to cause contact sensitization in

From the Columbia University College of Physicians and Surgeons^a and Department of Dermatology, Columbia University Medical Center.^b

Columbia University is supported by the National Heart, Lung, and Blood Institute/National Institutes of Health Ruth L. Kirschstein National Research Service Award Short-Term Institutional Research Training Grant (T35).

Conflicts of interest: None declared.

Accepted for publication March 5, 2013.

Reprints not available from the authors.

Correspondence to: Donald V. Belsito, MD, Department of Dermatology, Columbia University Medical Center, Herbert Irving Pavilion, Room 1231, 161 Fort Washington, New York, NY 10032. E-mail: dvb2108@columbia.edu.

Published online April 11, 2013.

0190-9622/\$36.00

© 2013 by the American Academy of Dermatology, Inc.

<http://dx.doi.org/10.1016/j.jaad.2013.03.012>

the general population, with an estimated prevalence of 8.6% worldwide.⁵

There is ongoing debate in the literature regarding the relationship between AD and contact dermatitis.⁶⁻⁸ A literature review by Spiewak⁶ found numerous contradictory statements on the relationship between atopy and contact dermatitis; several studies have found that patients with AD have reduced contact sensitivity to allergens,⁸⁻¹² whereas others have found a positive relationship between AD and contact sensitivity.^{7,13-15} Still other studies, including that by Spiewak,⁶ have found atopy and contact dermatitis to be independent.¹⁶⁻¹⁸

Those who have found a positive relationship between AD and CDTH suggest that the dry, inflamed skin of people with atopic eczema requires frequent application of emollients, corticosteroids, and antibacterial creams that may result in contact sensitization to those allergens.¹⁹ In addition, disruption of the cutaneous barrier, as is present in AD, may promote the inflammatory conditions that lead to contact sensitization. Those whose findings support that people with AD are less likely to develop delayed-type hypersensitivity response believe that, in AD, the balance between Th1 and Th2 immune responses is skewed in favor of Th2, thus decreasing the ability of the immune system to mount a Th1-mediated, delayed hypersensitivity response to contact allergens.²⁰

There have also been reports that certain allergens are more likely to generate CDTH responses in atopic populations.^{19,21,22} Danish¹⁹ and German²² studies have noted that fragrances were statistically more likely to result in a positive patch test in people with AD. Thus, it is often suggested that atopic patients minimize their exposure to fragrances.

Given the current understanding of the relationship between AD and CDTH, we were curious to examine 3 areas of study. First, we aimed to further assess the relationship between AD and CDTH as measured by positive allergic patch test reactions, regardless of relevance, in patients undergoing patch testing for suspected allergic contact dermatitis. Second, we wanted to determine the most common allergens to generate positive patch tests among those with and without AD in our study population. And finally, we

assessed trends in the incidence of positive reactions to allergens found in patients with and without AD.

METHODS

Between July 1, 1994, and June 30, 2012, a total of 2305 patients, who presented with a clinical suspicion of allergic contact dermatitis, underwent

patch testing to the NACDG's standard allergen series by the senior author in both Kansas City, KS, and New York, NY. Before patch testing, all patients completed a standardized questionnaire regarding demographic, medical, and occupational data. Atopic status (dermatitis, asthma, hay fever) was assessed in all patients; the diagnosis of AD was established using the criteria of Hanifin and Rajka.²³

Patients were patch tested in a standardized manner using Finn Chambers (Epitest Ltd Oy, Tuusula, Finland) on Scanpor tape (Bard Medical, Covington, GA).²⁴ Patch tests

were applied to areas of the back free of dermatitis. In general, patients with active dermatitis involving 25% or more of body surface area were not patch tested because of the enhanced possibility of false-positive ("angry back") reactions. Test allergens were purchased from Chemotechnique Diagnostics AB, Malmö, Sweden (1994–2007), or from SmartPractice, Calgary, Alberta, Canada (2008–2012). Allergens were applied on Mondays, and patients were examined at days 2 and 4 after placement. Reactions were assessed based on morphology as previously described.²⁴ Reactions scored as a 1+, 2+, or 3+ were considered a positive allergic response.

All deidentified Health Insurance Portability and Accountability Act–compliant data were entered, retrieved, and evaluated using a computer database (Access 2010, Microsoft Corp, Seattle, WA) and this study was therefore considered exempt from institutional review board approval at Columbia University Medical Center, New York, NY. The incidence of contact sensitization to any allergen, to metal allergens (nickel sulfate, cobalt chloride, and potassium dichromate), and to fragrances (fragrance mix I and balsam of Peru) among patients with AD (n = 297) and without AD (n = 2008) was assessed. A χ^2 test was conducted to test whether the difference

CAPSULE SUMMARY

- The relationship between atopic dermatitis and cutaneous delayed-type hypersensitivity is currently in dispute.
- We found that, compared with nonatopic patients, those with atopic dermatitis have a higher incidence of at least 1 positive patch test reaction to a standard screening series. Atopic individuals were significantly more likely to exhibit cutaneous delayed-type hypersensitivity to metal allergens.
- Health care practitioners should counsel atopic patients to minimize cutaneous contact with metals and, especially, to avoid piercing and tattooing.

between observed and expected frequencies was statistically significant, using statistical software (R, Version 2.15.1, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Of the 2305 patients patch tested, 12.89% ($n = 297$) had a history of AD. Among the 825 men enrolled in the study, the incidence of AD was 8.61% ($n = 71$) and, in the female population of 1478, the incidence of AD was 15.29% ($n = 226$).

As seen in Fig 1, among those with no history of AD ($n = 2008$), 64.49% had a positive patch test response to at least 1 allergen, whereas 71.72% of those with a history of AD ($n = 297$) did ($P = .017$). Subanalysis by gender was not statistically significant, most likely because of smaller sample sets. The top 10 most frequent allergens in both the atopic and nonatopic groups are delineated in Table I.

When examining common allergens in groups of patients with and without a history of AD, there were no statistically significant differences between these 2 groups in the incidence of positive responses to either fragrance mix I ($P = .72$) or balsam of Peru ($P = .78$). These results held true when analyzed by gender (Table II).

However, 1 striking finding was the significantly higher incidence of positive patch test reactions to metal allergens among those patients with a history of AD than in those without (Table II and Fig 2). When considering nickel sulfate, 21.21% ($n = 63$) of patients with AD had a positive response, whereas only 11.95% ($n = 240$) of patients with no history of AD had a positive response ($P = .00002$). Because nickel allergy is more common in women, we analyzed by gender and the above finding held true in both men ($P = .002$) and women ($P = .006$). Patients with a history of AD were also significantly more likely to have a positive response to the metal allergens cobalt chloride and potassium dichromate. For cobalt chloride, 14.14% ($n = 42$) of people with a history of AD had a positive reaction, whereas 7.72% ($n = 155$) of those with no history of AD did ($P = .0003$). For potassium dichromate, 7.07% ($n = 21$) of patients with AD tested positive, whereas 3.34% ($n = 67$) of patients with no history of AD had a positive reaction ($P = .003$). In total, all findings proved significant when stratified by gender except that for cobalt allergy in men, where the data lost statistical power because of the relatively small number of men with AD included in the study (Table II).

DISCUSSION

In a prior study,¹¹ patients with severe AD were less likely sensitized to dinitrochlorobenzene than

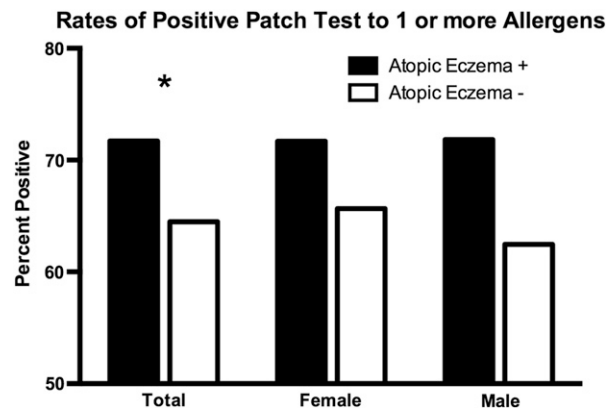


Fig 1. Cutaneous delayed-type hypersensitivity. Percentage of atopic and nonatopic patients who had positive patch test reaction to any allergen during testing. * $P < .05$.

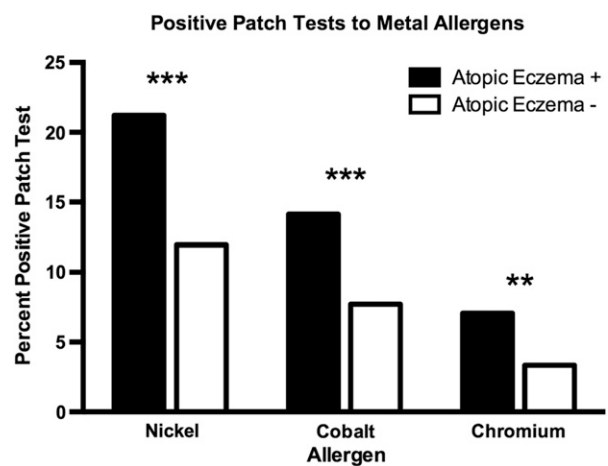


Fig 2. Cutaneous delayed-type hypersensitivity to metals. Percentage of patients testing positive to metal allergens in atopic and nonatopic groups. ** $P < .005$. *** $P < .0005$.

those with moderate or mild disease. In this study, of 20 patients with severe or moderate disease unresponsive to the initial dinitrochlorobenzene challenge, 18 reacted to a second challenge when the dermatitis was well controlled. In contrast, our data, collected from individuals with dermatitis covering 25% or less of body surface area, show that persons with a history of AD are significantly more likely than the non-AD population to have a positive allergic patch test reaction to at least 1 allergen. Thus, although the induction of CDTH (at least to dinitrochlorobenzene, a potent allergen) may be dampened in atopics,^{8,11} in the aggregate, patients with AD are more likely than nonatopic patients to acquire and manifest CDTH responses over time.

Our findings of an increased incidence of CDTH in atopic patients are consistent with the findings of Gittler et al²⁵ in their recent exhaustive review of the literature. As outlined by these authors, there are

Table I. Top 10 most frequently positive patch test reactions in nonatopic and atopic populations and their prevalence

No history of atopic eczema (n = 2008)		History of atopic eczema (n = 297)	
Allergen	%	Allergen	%
1 Nickel sulfate	11.95	Nickel sulfate	21.21
2 Fragrance mix I	8.62	Cobalt chloride	14.14
3 Bacitracin	8.22	Quaternium-15	13.13
4 Quaternium-15	8.12	Fragrance mix I	9.43
5 Cobalt chloride	7.72	Bacitracin	9.09
6 Balsam of Peru	7.37	Formaldehyde	8.08
7 Neomycin sulfate	6.87	p-Phenylenediamine	7.41
8 Formaldehyde	6.47	Thiuram mix	7.41
9 Thimerosal	5.23	Potassium dichromate	7.07
10 p-Phenylenediamine	4.68	Neomycin sulfate	7.07

Table II. Common metal and fragrance allergens and their rates of positive patch tests among atopic and nonatopic populations, stratified by gender

	Atopic eczema +		Atopic eczema –		P value
	No.	%	No.	%	
Nickel sulfate	63/297	21.21	240/2008	11.95	.00002
Female	49/226	21.68	178/1252	14.22	.006
Male	14/71	19.72	61/754	8.09	.002
Cobalt chloride	42/297	14.14	155/2008	7.72	.0003
Female	33/226	14.6	105/1252	8.39	.005
Male	9/71	12.68	50/754	6.63	.099
Potassium dichromate	21/297	7.07	67/2008	3.34	.003
Female	11/226	4.87	23/1252	1.84	.011
Male	10/71	14.08	44/754	5.84	.046
Fragrance mix I	28/297	9.43	173/2008	8.62	.72
Female	24/226	10.62	105/1252	8.39	.33
Male	4/71	5.63	68/754	9.02	.46
Balsam of Peru	20/297	6.73	148/2008	7.37	.78
Female	14/226	6.19	86/1252	6.87	.82
Male	6/71	8.45	62/754	8.22	1

multiple possible mechanisms accounting for enhanced CDTH in atopic patients including barrier disruption, cutaneous/systemic immunologic abnormalities, and genetic factors. However, which of these factors, or what combination thereof, might account for our most striking finding that atopics are more likely than nonatopics to react to metal allergens is not known.

It has previously been found that atopic patients have higher rates of sensitization to chromium²¹; however, the trend has never before been extended to cobalt. Regarding reactions to nickel, although mutations in the filaggrin (FLG) gene have been

associated with nickel allergy,^{26,27} a correlation between AD and CDTH to nickel has been previously demonstrated only in unpierced women.¹⁹ Thus, our findings that atopic individuals have a higher incidence of CDTH to chromium, nickel, and cobalt than non-AD patients are novel. Nonetheless, it should be noted that, in a study of 89 Tunisian atopic patients (also classified using the criteria of Hanifin and Rajka²³) patch tested to the European standard series, the top 3 allergens were nickel (24.7%), chromium (7.9%), and cobalt (7.9%).²⁸ Unfortunately, the rate of reactions to these allergens among the nonatopic patients tested at this Tunisian center is unknown.

Our finding that AD was associated with contact sensitization to metal allergens can be explained in several ways: (1) atopics display a genetic predisposition to develop a delayed-type hypersensitivity response to metals via Toll-like receptor (TLR) dysfunction; (2) defects in FLG in people with AD allow for enhanced penetration of metal allergens below the stratum corneum; or (3) increased exposure to metal allergens in people with AD in the United States leads to higher rates of sensitization.

Polymorphisms in TLRs may explain the link between AD and reactivity to metals. In 2010, Schmidt et al²⁹ showed that nickel directly activates TLR4 to induce contact hypersensitivity to nickel. Studies in mice show that TLR4 inhibitors dampen the allergic contact response.³⁰ In addition, TLRs 2 and 9 have been implicated in the development of AD.^{31,32} Further investigation is necessary to examine the possibility of alterations in TLR function in persons with atopic disease that may lead to their increased susceptibility to develop nickel (and other metal) allergies.

Enhanced sensitivity to metals in atopics may also be related to reports that loss-of-function mutations in FLG, leading to impaired skin barrier function, are major predisposing factors to the development of AD. FLG, a protein whose function is to aid in terminal differentiation of the epidermis and formation of the skin barrier, was found to be mutated in 17% to 50% of people with atopic eczema.³³ In 1 study,²⁷ loss-of-function mutations in FLG were associated with contact sensitivity to nickel, and intolerance to costume jewelry, which often contains high levels of nickel. However, increased rates of contact sensitivity to 24 other allergens were not observed in people with FLG mutations in that study.²⁷ The exact mechanism by which FLG interacts with nickel in the stratum corneum is unknown, however it has been speculated that the histidine-rich polypeptide FLG chelates nickel in the epidermis and creates a reservoir

that prevents free ions from permeating the skin, where they may induce sensitization or elicitation.^{26,34,35} This mechanism would explain the findings of the previous study in which loss-of-function FLG mutations were associated with nickel contact sensitivity,²⁷ and possibly to our findings that AD predisposes to other metal allergens, specifically cobalt and chromium. However, this model is complicated by the possibility of multiple routes of exposure to nickel, including some common routes, such as piercings and tattoos that bypass the epidermal barrier altogether.¹⁹

Regarding our last hypothesis that atopic patients in the United States were more likely to have had nickel and other metal exposures, there are several possibilities. The most obvious would be piercings, as it is well established that people with piercings are at increased risk of developing metal allergy.³⁶ Although it remains a possibility that the increased incidence of nickel allergy in patients with AD is a result of a higher rate of piercing in this population, we think this to be highly unlikely. There are no data to suggest that AD is correlated with piercings; however, we did not collect data on piercings and therefore cannot control for this factor. Nonetheless, in their study, Thyssen et al¹⁹ found no increased incidence of nickel hypersensitivity in atopic patients except when they specifically looked at women without piercings, a subgroup in which atopic patients had a higher incidence of sensitization to nickel.

It should be pointed out that, as opposed to countries in the European Union, the United States does not regulate consumer exposure to nickel. In 1990, the Danish government began to regulate consumer nickel exposure as a response to the increasing nickel allergy problem. In 1994, the European Union Nickel Directive was passed, a regulation that was based on the Danish nickel regulations, which required that items in direct and prolonged contact with the skin could not release more than 0.5 μg nickel/ cm^2/wk . As a result, the incidence of nickel allergy in Europe has significantly decreased over the past decade.³⁷ Thus, the incidence of CDTH to nickel (and other metals) in atopic patients in the United States and those in Europe (from which most of the available data come) is not necessarily comparable.

The same reasoning regarding differences in population exposure may apply to our findings that, in the United States, atopics were no more likely to demonstrate CDTH to fragrances than non-atopics, a finding in contrast to that reported by the Danes¹⁹ and Germans.²² Many cases of fragrance allergy are thought to arise from use of underarm

deodorants. It has been reported that Europeans prefer deodorants, whereas US consumers favor antiperspirants with lower fragrance levels.³⁸ These different exposure patterns were thought to account for the significantly lower rate of allergic reactions to 3- and 4-(4-hydroxy-4-methylpentyl)cyclohexene-1-carboxaldehyde among North Americans than that observed throughout Europe.³⁸

To conclude, our patients with AD are more likely to have a CDTH reaction than those without. Furthermore, although we did not find atopics to be more likely to react to fragrances, as has been previously reported, we did observe a statistically increased incidence of reactivity to all 3 of the metal allergens that have been consistently on the NACDG standard series from 1994 through 2012. Although the reason for this enhanced susceptibility requires further study, we strongly recommend that health care practitioners counsel their atopic patients to minimize cutaneous contact with metals, and to avoid piercing and tattooing.

REFERENCES

1. Moehrenschrager M, Darsow U, Schnopp C, Ring J. Atopic eczema: what's new? *J Eur Acad Dermatol Venereol* 2006;20: 503-11.
2. Bieber T. Atopic dermatitis. *Ann Dermatol* 2010;22:125-37.
3. Kapoor R, Menon C, Hoffstad O, Bilker W, Leclerc P, Margolis DJ. The prevalence of atopic triad in children with physician-confirmed atopic dermatitis. *J Am Acad Dermatol* 2008;58:68-73.
4. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol* 2003;112:S118-27.
5. Thyssen JP, Linneberg A, Menne T, Johansen JD. The epidemiology of contact allergy in the general population—prevalence and main findings. *Contact Dermatitis* 2007;57: 287-99.
6. Spiewak R. Atopy and contact hypersensitivity: a reassessment of the relationship using objective measures. *Ann Allergy Asthma Immunol* 2005;95:61-5.
7. Herro EM, Matiz C, Sullivan K, Hamann C, Jacob SE. Frequency of contact allergens in pediatric patients with atopic dermatitis. *J Clin Aesthet Dermatol* 2011;4:39-41.
8. Rees J, Friedmann PS, Matthews JN. Contact sensitivity to dinitrochlorobenzene is impaired in atopic subjects: controversy revisited. *Arch Dermatol* 1990;126:1173-5.
9. Rudzki E, Grzywa Z. Contact sensitivity in atopic dermatitis. *Contact Dermatitis* 1975;1:285-7.
10. Jones HE, Lewis CW, McMarlin SL. Allergic contact sensitivity in atopic dermatitis. *Arch Dermatol* 1973;107:217-22.
11. Uehara M, Sawai T. A longitudinal study of contact sensitivity in patients with atopic dermatitis. *Arch Dermatol* 1989;125: 366-8.
12. de Groot AC. The frequency of contact dermatitis in atopic patients with dermatitis. *Contact Dermatitis* 1990;22:273-7.
13. Manzini BM, Ferdani G, Simonetti V, Donini M, Seidenari S. Contact sensitization in children. *Pediatr Dermatol* 1998;15: 12-7.
14. Dotterud LK, Falk ES. Contact allergy in relation to hand eczema and atopic diseases in north Norwegian school-children. *Acta Paediatr* 1995;84:402-6.

15. Forsbeck M, Hovmark A, Skog E. Patch testing, tuberculin testing and sensitization with dinitrochlorobenzene and nitro-sodimethylaniline of patients with atopic dermatitis. *Acta Derm Venereol* 1976;56:135-8.
16. Lammintausta K, Kalimo K, Havu VK. Contact allergy in atopics, who perform wet work in hospital. *Derm Beruf Umwelt* 1982;30:184-8.
17. Mortz CG, Lauritsen JM, Bindslev-Jensen C, Andersen KE. Contact allergy and allergic contact dermatitis in adolescents: prevalence measures and associations; the Odense adolescence cohort study on atopic diseases and dermatitis (TOACS). *Acta Derm Venereol* 2002;82:352-8.
18. Rystedt I. Contact sensitivity in adults with atopic dermatitis in childhood. *Contact Dermatitis* 1985;13:1-8.
19. Thyssen JP, Linneberg A, Engkilde K, Menné T, Johansen JD. Contact sensitization to common haptens is associated with atopic dermatitis: new insight. *Br J Dermatol* 2012;166:1255-61.
20. Spergel JM. From atopic dermatitis to asthma: the atopic march. *Ann Allergy Asthma Immunol* 2010;105:99-106.
21. Landeck L, Schalock P, Baden L, González E. Contact sensitization in 172 atopic subjects. *Int J Dermatol* 2011;50:806-10.
22. Heine G, Schnuch A, Uter W, Worm M. Type IV sensitization profile of individuals with atopic eczema: results from the information network of departments of dermatology (IVDK) and the German contact dermatitis research group (DKG). *Allergy* 2006;61:611-6.
23. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980;92:44-7.
24. Storrs FJ, Rosenthal LE, Adams RM, Clendenning W, Emmett EA, Fischer AA, et al. Prevalence and relevance of allergic reactions in patients patch tested in North America—1984 to 1985. *J Am Acad Dermatol* 1989;20:1038-45.
25. Gittler JK, Krueger JG, Guttman-Yassky E. Atopic dermatitis results in intrinsic barrier and immune abnormalities: implications for contact dermatitis. *J Allergy Clin Immunol* 2013;131:300-13.
26. Thyssen JP, Carlsen BC, Menne T. Nickel sensitization, hand eczema, and loss-of-function mutations in the filaggrin gene. *Dermatitis* 2008;19:303-7.
27. Novak N, Baurecht H, Schäfer T. Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. *J Invest Dermatol* 2008;128:1430-5.
28. Belhadjali H, Mohamed M, Youssef M, Mandhouj S, Chakroun M, Zili J. Contact sensitization in atopic dermatitis: results of a prospective study of 89 cases in Tunisia. *Contact Dermatitis* 2008;58:188-9.
29. Schmidt M, Raghavan B, Müller V. Crucial role for human Toll-like receptor 4 in the development of contact allergy to nickel. *Nat Immunol* 2010;11:814-9.
30. Di Nardo A, Braff MH, Taylor KR, Na C, Granstein RD, McInturff JE, et al. Cathelicidin antimicrobial peptides block dendritic cell TLR4 activation and allergic contact sensitization. *J Immunol* 2007;178:1829-34.
31. Lesiak A, Smolewski P, Sobolewska-Sztychny D, Sysa-Jedrzejowska A, Narbutt J. The role of T-regulatory cells and toll-like receptors 2 and 4 in atopic dermatitis. *Scand J Immunol* 2012;76:405-10.
32. Novak N, Yu CF, Bussmann C, Maintz L, Peng WM, Hart J, et al. Putative association of a TLR9 promoter polymorphism with atopic eczema. *Allergy* 2007;62:766-72.
33. Palmer CN, Irvine AD, Terron-Kwiatkowski A, Zhao Y, Liao H, Lee SP, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006;38:441-6.
34. Fullerton A, Hoelgaard A. Binding of nickel to human epidermis in vitro. *Br J Dermatol* 1988;119:675-82.
35. Fullerton A, Andersen JR, Hoelgaard A. Permeation of nickel through human skin in vitro—effect of vehicles. *Br J Dermatol* 1988;118:509-16.
36. Brandão MH, Gontijo B, Girundi MA, de Castro MC. Ear piercing as a risk factor for contact allergy to nickel. *J Pediatr (Rio J)* 2010;86:149-54.
37. Thyssen JP, Uter W, McFadden J, Menné T, Spiewak R, Vigan M, et al. The EU nickel directive revisited—future steps toward better protection against nickel allergy. *Contact Dermatitis* 2011;64:121-5.
38. Belsito DV, Fowler JF, Sasseeville D, Marks JG, Deleo VA, Storrs FJ. Delayed-type hypersensitivity to fragrance materials in a select North American population. *Dermatitis* 2006;17:23-8.