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Contact sensitization to common haptens is associated with atopic dermatitis: new insight

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What is already known about this topic?

- Experimental studies have clearly shown that individuals with atopic dermatitis have suppressed contact sensitivity due to their disease.
- General population and clinical studies have found diverging outcomes, some suggesting a positive association between atopic dermatitis and contact sensitization.
- Nickel sensitization and filaggrin mutations have been positively associated in two studies.

What does this study add?

- By limiting potential bias from contact allergens that may cause systemic allergen exposure, a strong and positive association between atopic dermatitis and contact sensitization was identified.
- Physicians should inform their patients about a potentially increased risk of sensitization.
- Patch testing should be considered at an early point in individuals with a history of atopic dermatitis and active disease.

Abstract

Introduction: It has been much debated whether atopic dermatitis is associated with contact sensitization since past findings have conflicted. A positive association might change our clinical practice.

Objective: To investigate the association between atopic dermatitis and contact sensitization taking the likely route of allergen exposure into account.

Materials and Methods: Questionnaire and clinical data from a cross-sectional study performed in a general population in Copenhagen. A total of 3202 (40.4%) 18-69 year olds were patch tested, filaggrin genotyped for 2282del4 and R501X and questioned about AD

Results: The variable “contact sensitization to common chemicals, but not nickel and thimerosal”, was significantly associated with atopic dermatitis (OR=2.53; CI95%=1.59-4.04). The higher prevalence of contact sensitization was mainly driven by fragrance chemicals. In a sub-analysis in non-pierced women, a positive association was also found for nickel sensitization. Nickel and thimerosal sensitization may introduce bias in data analysis since these allergies often develop following skin piercing where the skin compartments are bypassed.

Discussion: We suspect that individuals with self-reported atopic dermatitis from this study mainly suffered from mild disease. However, clinicians should be aware of increased levels of contact sensitization in individuals with atopic dermatitis. Patch testing should therefore be considered at an early point in individuals with a history of atopic dermatitis and active disease. The fundamental relationship between atopic disease and environmental chemical exposure may be of a more complex and intimate nature than previously supposed.

Key words: atopic dermatitis, contact sensitization, general population, epidemiology

INTRODUCTION

Atopic dermatitis (AD) is a common skin condition characterized by dry and itchy skin with chronic or recurrent episodes of dermatitis located at typical anatomical sites. Despite the fact that AD is mainly a disease of childhood, it may sometimes persist or have a late onset. Contact sensitization is on the other hand prevalent in both children and adults reaching up to 20%^{1;2}. It has been debated whether AD is associated with contact sensitization since past findings have conflicted^{3;4}. Thus, clinical studies have shown that exposure to contact allergens used in topical products may result in contact sensitization^{5;6} but sensitization trials have shown that especially moderate to severe atopic dermatitis is inversely associated with contact sensitization probably since cell mediated immunity is suppressed in AD⁷⁻⁹ as also seen in other inflammatory skin diseases^{10;11}. To some surprise, a German general population study recently showed a positive association between filaggrin mutations (which have been shown to be strongly associated with AD) and contact sensitization to nickel¹²; a finding that was partly confirmed by our group since we could only find a positive association in the very few women without ear piercing.¹³ Also, using the same general population, we showed that the prevalence of contact sensitization to certain chemicals used in topical products, e.g. fragrances, ethylenediamine, and neomycin was higher in individuals with AD and filaggrin mutation status when compared to filaggrin non-mutation carriers without AD⁵. The recent findings on the association between filaggrin and contact sensitization have made us realize that the likely route of exposure should be taking into account when evaluating the association between these disorders to limit bias. Hence, sensitization to contact allergens like nickel and thimerosal may not only be induced following skin exposure to these allergens, but also following ear piercing with nickel releasing jewellery and injections with vaccines containing thimerosal. We suspect that results from past epidemiological studies on the association between AD and contact sensitization might have been biased by inclusion of nickel sensitization in the analyses since nickel is by far the most common contact allergen and since a majority of women are sensitized following ear piercing. Therefore, we use general population patch test data in an attempt to re-investigate the association between AD and contact sensitization taking these considerations into account.

MATERIALS AND METHODS

Study population

Between June 2006 and May 2008, a cross-sectional study on general health was performed in the general population in Copenhagen. A random sample of 7931 subjects aged 18-69 years was obtained from the Danish Central Personal Register. All were adults with Danish citizenship and born in Denmark. A section of the health survey focused on skin health, in particular hand eczema and contact sensitization. A total of 3471 (43.7%) subjects participated in a general health examination at the research facility. Some 3460 were patch tested, 3335 were successfully filaggrin genotyped and 3329 answered the questions on AD. Selecting participants with data on each of these variables reduced the sample size to 3202 (40.4%). Hence, these participants were all patch tested, filaggrin genotyped for 2282del4 and R501X and answered the questions on AD. The participation rate was higher among older age-groups than among younger age-groups in both genders¹⁴. The Ethical Committee of Copenhagen County approved the study (KA-20060011). A written informed consent form was obtained from all participants prior to the beginning of the study.

Questionnaire

All participants were mailed a standard invitation letter and a questionnaire about health, lifestyle, and socio-economic factors. Among other questions, participants were asked “have you ever had your ears pierced?”. AD was defined by the U.K. Working Party's diagnostic criteria for AD as a self-reported history of an itchy skin condition plus a minimum of two of four minor criteria¹⁵. The questionnaire was returned prior to patch testing.

Measurement of Immunoglobulin E antibodies

Venous blood was taken on the day of examination. The serum was then recovered by centrifugation at 3000 r.p.m. for 10 min and stored frozen until analysis for IgE specific to birch, grass (timothy), cat, and mite (*Dermatophagoides pteronyssinus*) with the ADVIA Centaur IgE antibody

assay system (Bayer Corporation)¹⁶. The analysis was judged to be positive if the measurement was in excess of 0.35 kU/l towards at least one of the four allergens tested.

Patch tests

Patch testing was performed by using the panel 1 and 2 of the standardized ready to apply TRUE-test (Mekos Laboratories, Hillerød, Denmark in 2006). Contact sensitization was defined as a positive (at least grade 1+ according to The International Contact Dermatitis Research Group (ICRDG) criteria¹⁷) patch test to at least one allergen or mixes of haptens on day 2. For details please refer to our previous publication².

Filaggrin genotyping

A venous blood sample was drawn. Regions covering the mutations R501X and 2282del4 of the filaggrin gene were amplified from genomic DNA by PCR, and the obtained DNA fragments were hybridized to both mutation specific and wildtype specific DNA-probes attached to fluorescent microbeads (Luminex, Austin, TX, USA). They were subsequently analyzed on a Bio-Plex 200 (Biorad, Hercules, CA, USA)¹³.

Statistical analysis

Characteristics of participants were compared using the chi-square (χ^2) test. Filaggrin mutation status was defined as subjects who were heterozygote, compound heterozygote or homozygote for mutations R501X or 2282del4¹³. Logistic regression models were performed with “AD” as the dependent variable, and with gender, age-group (“18-35”, “36-55”, “56-69” years), filaggrin genotype (“wildtype”, “null”), specific IgE, and contact sensitization as the independent variables. In these models, tests for interaction between contact sensitization and, respectively, filaggrin mutation status, gender and age group were performed by using a log-likelihood ratio test. Since cross-sectional studies can not determine cause and effect, we chose this approach allowing repeated association analyses with different contact allergen variables. Associations were expressed as

odds ratios (ORs) with confidence intervals (CIs) of 95%. Data analyses were performed using the Statistical Products and Service Solutions package (SPSS Inc., Chicago, IL, USA) for windows (release 15.0).

RESULTS

Patch test results from 3 202 participants aged 18-69 years (1 774 women and 1 428 men) who responded to the questions on AD are presented in table 1. A total of 316 (9.9%) positive patch test reactions were identified and a higher proportion was observed in women (14.1%) than in men (4.6%). The most common contact allergens were nickel (5.9%), fragrance mix I (1.6%), colophony (0.6%) and thimerosal (0.5%). In crude data analysis, the prevalence of contact sensitization to at least one allergen was more common in participants who reported AD (13.6%) when compared to those who denied AD (9.5%) ($p=0.018$). A similar observation was made in female (16.3% vs 13.8%, $p=0.30$) and male (6.6% vs 4.5%, $p=0.36$) participants albeit the difference was not statistically significant. A higher prevalence of contact sensitization in participants with AD was observed for the majority of allergens in both genders (table 1). In a sub-analysis of 308 women who denied ear piercing, AD was positively associated with nickel sensitization (5.3% vs. 2.8%, $OR=1.95$; $CI_{95\%}=0.23-16.46$).

The prevalence of AD in different groups is shown in table 2. Since nickel and thimerosal exposure are often associated with skin penetration, e.g. ear and body-piercing as well as vaccinations, we also made contact sensitization variables without nickel and thimerosal sensitization to prevent potential confounding by exposures where the skin compartments had been by-passed (table 1 and 2). Female gender, young age, filaggrin loss-of-function mutations, and specific IgE were positively associated with AD. Logistic regression models performed with “AD” as the dependent variable showed a positive but non-significant association with contact sensitization to at least one allergen ($OR=1.20$; $CI_{95\%}=0.83-1.71$). Tests for interaction between contact sensitization and, respectively, filaggrin mutation status, gender and age group were not statistically significant; hence the p-values were $p=0.81$ for filaggrin mutation status, $p=0.78$ for gender and $p=0.25$ for age group. Similar results were obtained when similar regression analyses were made with “contact sensitization

to at least one allergen but not nickel and thimerosal” instead of “contact sensitization to at least one allergen”. This suggests that the effect of contact sensitization on the prevalence of AD did not depend significantly on filaggrin loss-of function mutation status, gender or on age group. In further adjusted data analyses, we found a positive and statistically significant association between contact sensitization to at least one allergen but not nickel and thimerosal and AD (OR=2.53; CI95%=1.59-4.04) whereas no association was found with nickel sensitization (OR=0.66; CI95%=0.39-1.12) (table 2).

DISCUSSION

Contact sensitization was strongly associated with AD in adults from a general population in Denmark. The association was similar in individuals with and without filaggrin mutations. Our finding was based on data analyses in which thimerosal and nickel sensitization were excluded since these contact allergies to a very high degree are caused by skin piercing due to vaccinations or insertion of ear posts ^{2,18}. In fact, we recently showed that the prevalence of thimerosal sensitization in a general population decreased from 3.5% in 1990 to 0.5% in 2006, probably due to removal of thimerosal from the national vaccination program ². If thimerosal and nickel contact sensitization are not addressed separately, they may introduce bias in the analyses as recently suggested by the “by-pass theory” ¹⁹. Nickel sensitization may also have been caused by excessive cutaneous exposure to e.g. non-piercing jewellery or clothing fasteners, but such exposures could not be established from our data. In a sub-analysis on women who denied ear piercing, a positive association was found between nickel sensitization and AD, again suggesting that AD and contact sensitization are positively associated in the general population. The higher prevalence of contact sensitization in individuals who reported AD was mainly caused by fragrance mix I, colophony, epoxy resin and ethylenediamine. However, a difference in favour of AD was observed for 13 chemicals whereas 7 chemicals had higher prevalence estimates in non-AD participants (table 1). The positive association did not depend on gender, age, filaggrin mutation status, or specific IgE suggesting that the higher prevalence was not associated with a subgroup of AD and that it was rather

a general trait. However, we recently showed that the prevalence of contact sensitization was higher in individuals with AD who also had filaggrin loss-of-function mutations when compared to individuals with AD and wild type filaggrin status ⁵; suggesting that the severity of disease caused by filaggrin mutations may increase the risk of contact sensitization.

From a clinical perspective, a positive association seems plausible as well as logical since individuals with AD suffer from dry and inflamed skin resulting in frequent application of (scented) emollients as well as topical corticosteroid and antibacterial creams. This may eventually result in contact sensitization and allergic contact dermatitis. It should however be underscored that cross-sectional studies are not able to determine cause and effect. Hence, it is possible that AD may not only result in contact sensitization but also that contact sensitization may result in AD in selected individuals or at least worsen the course. In the 1950's, cutaneous nickel exposure was very high in females who wore nickel plated stocking suspenders. Calnan noted that 75% of 400 female nickel allergic dermatitis patients, of whom 95% had stocking suspender dermatitis, developed "haematogenous" spread of dermatitis affecting the elbow flexures, eyelids, sides of neck and face and inner aspects of the thighs ²⁰. This clinical picture is reminiscent of AD, but Calnan showed that only 4 patients had previously suffered from AD but that 15% and 22% had a personal history or a family history of atopy, respectively. Moreover, an Australian group recently showed that occupational contact dermatitis could precipitate a flare of more generalized eczema, in a pattern consistent with atopic dermatitis ²¹. The increase of AD in Western societies might to some degree have been influenced by exposure to chemicals including contact allergens. Also, systemic contact dermatitis may mimic AD resulting in incorrect therapy.

The positive association between AD and contact sensitization in our study was mainly driven by fragrance chemicals. This finding conflicts with previous findings based on patch tested dermatitis patients. Hence, a British study including 34 072 patients ²² and a German study including 59 298 patients ²³ found no difference in the prevalence of fragrance sensitization in dermatitis patients with or without AD. However, another large German study found a significantly higher prevalence of fragrance mix I sensitization in patients with AD when compared to controls ²⁴. A

Swedish study also found a high prevalence of fragrance sensitization in patients with AD ²⁵. A reasonable explanation for the different outcomes can be that bias might have affected the outcome of patient based studies since they have all been conducted on dermatitis patients that were patch tested due to suspicion of allergic contact dermatitis. Hence, both cases and controls had a medical history suggesting exposure and inflamed skin resulting in impaired skin barrier functions and the need for topical therapy including emollients. Experimental sensitization trials have shown that challenge with the experimental sensitizer dinitrochlorobenzene (DNCB) was positive in 33% of 24 patients with severe AD, 95% of 86 patients with moderate AD and in 100% of 40 patients with mild AD ⁹.

Reduced reactivity in patients with severe dermatitis might be explained by increased T-helper cell 2 deviation of the immune system as opposed to T-helper cell 1 deviation as traditionally observed in contact sensitization. When re-challenge was performed in patients who did not react on the first occasion, 18 reacted when dermatitis was well controlled suggesting that a possible inverse association between AD and contact sensitization is mainly associated with severe disease. A Swedish follow-up study, on the other hand, found a significantly higher prevalence of contact sensitization in patients who had AD after age 15-years when compared to controls with healed AD before age 15 years ²⁵. Furthermore, a British research group lead by John McFadden and Ian Kimber have proposed the “haptten-atopy” hypothesis which postulates that an increased exposure to hapten (and irritant) chemicals in the personal environment, particularly during pregnancy and the first year of life when there is a Th2 bias, may predispose to subsequent development of atopic disease in the child ²⁶. They suggest that the increased exposure over the last 50 years to these chemicals in agents such as skin care products, processed foods and drugs may have contributed to the correlating increase in atopic disease over the same period. Supporting observations include animal models of atopic dermatitis based on repeated application of haptens ²⁷ and increased incidence of atopic disease in children born to women with specific occupations during pregnancy which are associated with high hapten/irritant exposure ²⁸. These observations underscore the complexity of this area and show that dermatitis severity as well as the timing and route of hapten exposure is critical for contact sensitization.

General population studies tend to be less biased than patient based studies and for this reason we believe our findings are highly reliable. Patch testing was only a small part of a 2-hour general health examination including focus on cardiovascular and respiratory diseases, diabetes, thyroid disorders, etc. No skin examination was provided. Despite a low participation rate, we do not suspect that participants with skin diseases were selected particularly for this study. Patch test readings were performed only on day 2 which might have resulted in false negative reactions but also led to irritant reactions (in particular against fragrance chemicals) that could have been incorrectly interpreted as positive ones. However, it should be noted that fragrance mix is also considered an early “reactor” offering reliable contact sensitization estimates already on day 2 ²⁹. There is some insight in the variation of patch test reactivity in individuals with and without AD. A German study suggested that patients with a predisposition to AD had more doubtful and irritant reactions when readings were performed on day 1 than controls ³⁰. Also, atopic individuals tended to have stronger reactions on day 3 to fragrances and formaldehyde. In our study, patch test interpretation was performed without information about AD status or previous skin complaints to limit bias and agreement was reached by experienced patch testers in a blinded manner. Since fragrance mix I only include some fragrance chemicals, sensitization to other important fragrances was missed. AD was defined by the UK Working Party’s Criteria offering a sensitivity and specificity of, respectively, 92% and 81% ¹⁵ making the AD diagnosis sufficiently reliable. In further support of our findings, a large Norwegian population based study recently found a positive and significant association between contact sensitization and AD, albeit not as strong as our association ³¹. Studies using our data approach strategy are clearly warranted to confirm these findings as are prospective studies conducted in children and adults. AD severity was not assessed in our study which is a limitation.

Clinicians should be aware of the association between AD and contact sensitization and patch testing should be considered at an early point in individuals with a history of AD and active dermatitis. Although no study so far has sufficiently examined how contact sensitization affects the course of dermatitis in individuals with AD ^{32;33}, contact allergen avoidance is expected to affect the prognosis positively. Emollient therapy for the primary prevention of AD and possibly food

sensitization and asthma was recently suggested ³⁴ since there is evidence to suggest that allergens penetrate filaggrin deficient skin and result in secondary sensitization and respiratory diseases ^{35;36}. Our data clearly indicate that treatment protocols and advice to parents should be based on hypo-allergenic products without fragrances and allergenic preservatives. There is a need for research establishing whether chemical exposure and/or contact sensitization may lead to AD.

Competing interests: The authors have no conflict of interest to disclose.

Contribution: Jacob Thyssen developed the research strategy together with Torkil Menné. Data analysis, manuscript preparation was done by Jacob Thyssen. Critical review was performed by all authors. All authors, except Kåre Engkilde, participated in designing the overall study as well as the questionnaire.

Allergens and mixtures of allergens	Typical sources of skin exposure	Positive reactions % (n)					
		Women		Men		Total	
		Atopic dermatitis					
		Yes % (n/n _{total})	No % (n/n _{total})	Yes % (n/n _{total})	No % (n/n _{total})	Yes % (n/n _{total})	No % (n/n _{total})
Nickel sulphate	Jewellery, buttons, belts, hair clasps	8.2 (18/219)	10.6 (158/1490)	0 (0/88)	1.1 (14/1288)	5.9 (18/307)	6.2 (172/2778)
Wool Alcohols	Lanolin is found in cosmetics	0 (0/228)	0 (0/1517)	0 (0/90)	0 (0/1314)	0 (0/318)	0 (0/2831)
Neomycin Sulfate	Aminoglycoside antibiotic used in medicated products	0.4 (1/227)	0.1 (1/1514)	0 (0/91)	0 (0/1315)	0.3 (1/318)	0.04 (1/2829)
Potassium Dichromate	Cement, leather, pigments, cutting oils	0.4 (1/227)	0.3 (4/1514)	0 (0/91)	0 (0/1317)	0.3 (1/318)	0.1 (4/2831)
Caine Mix	Local anaesthetics	0 (0/228)	0.1 (2/1509)	0 (0/91)	0 (0/1318)	0 (0/319)	0.07 (2/2827)
Fragrance Mix	Cosmetics, perfumes, toys	3.9 (9/228)	1.3 (20/1496)	2.2 (2/91)	1.5 (19/1305)	3.4 (11/319)	1.4 (39/2801)
Colophony	Cosmetics	0.9 (2/220)	0.5 (8/1493)	2.3 (2/87)	0.5 (7/1298)	1.3 (4/307)	0.5 (15/2791)
Epoxy Resin	Glues and adhesives	1.4 (3/222)	0.2 (3/1506)	2.3 (2/88)	0.8 (10/1313)	1.6 (5/310)	0.5 (13/2819)
Quinoline mix	Antimicrobials used in medicated creams	0 (0/223)	0 (0/1505)	0 (0/90)	0.1 (1/1313)	0 (0/313)	0.04 (1/2818)
myroxylon Pereirae	Cosmetics, plasters and adhesives	0 (0/221)	0.1 (2/1501)	0 (0/90)	0.1 (1/1308)	0 (0/311)	0.1 (3/2809)
Ethylenediamine Dihydrochloride	Topical corticosteroid and antibiotic creams	1.8 (4/220)	0.1 (1/1482)	0 (0/88)	0.2 (2/1299)	1.3 (4/308)	0.1 (3/2781)
Cobalt Dichloride	Jewellery, pigments, implants	0.5 (1/216)	0.3 (5/1458)	0 (0/89)	0.1 (1/1277)	0.3 (1/305)	0.2 (6/2735)
p-tert-Butylphenol Formaldehyde Resin	Glues and adhesives. Typical exposure is from shoes.	0.5 (1/220)	0 (0/1487)	0 (0/88)	0 (0/1283)	0.3 (1/308)	0 (0/2770)
Paraben Mix	Preservative used in cosmetics and occupational products	0 (0/226)	0 (0/1494)	0 (0/88)	0 (0/1306)	0 (0/314)	0 (0/2800)
Carba Mix	Rubber gloves, clothing, sports gear	0 (0/227)	0.1 (2/1491)	0 (0/88)	0.1 (1/1302)	0 (0/315)	0.1 (3/2793)
Black Rubber Mix	Black rubber products	0.4 (1/228)	0.2 (3/1499)	0 (0/88)	0 (0/1307)	0.3 (1/316)	0.1 (3/2806)
Cl+ Me- Isothiazolinone (MCI/MI)	Preservative used in cosmetics and occupational products	0.4 /1/230)	0.1 (1/1488)	0 (0/88)	0.2 (3/1296)	0.3 (1/318)	0.1 (4/2784)
Quaternium-15	Preservative used in cosmetics and occupational products	0 (0/224)	0.1 (2/1459)	0 (0/85)	0.2 (2/1271)	0 (0/309)	0.1 (4/2730)
Mercaptobenzothiazole	Rubber gloves, clothing, sports gear	0 (0/223)	0 (0/1494)	0 (0/87)	0 (0/1287)	0 (0/310)	0 (0/2781)
p-Phenylenediamine	Black hair dye, henna tattoos	0.4 (1/231)	0.1 (2/1519)	0 (0/90)	0 (0/1314)	0.3 (1/321)	0.1 (2/2833)
Formaldehyde	Preservative used in cosmetics and occupational products	0.4 (1/230)	0.2 (3/1511)	0 (0/90)	0.2 (2/1316)	0.3 (1/320)	0.2 (5/2827)
Mercapto Mix	Rubber gloves, clothing, sports gear	0 (0/232)	0 (0/1510)	0 (0/90)	0 (0/1310)	0 (0/322)	0 (0/2820)
Thimerosal	Vaccines and some eye and ear medication	0.9 (2/231)	0.6 (9/1504)	0 (0/88)	0.4 (5/1300)	0.6 (2/319)	0.5 (14/2804)

Thiuram Mix	Rubber gloves, clothing, sports gear	0 (0/229)	0.2 (3/1490)	0 (0/86)	0 (0/1292)	0 (0/315)	0.1 (3/2782)
At least one allergen but not nickel and thimerosal		9.9 (23/233)	3.8 (58/1541)	6.6 (6/91)	2.9 (39/1337)	9.0 (29/324)	3.4 (97/2878)
At least one allergen		16.3 (38/233)	13.8 (212/1541)	6.6 (6/91)	4.5 (60/1337)	13.6 (44/324)	9.5 (272/2878)

Table 1. The prevalence of contact sensitization to common haptens in Danish 18-69 year old adults from the general population in Copenhagen stratified by self-reported atopic dermatitis.

Table 2. Logistic regression analysis with the outcome “atopic dermatitis”¶ and with different explanatory variables performed in 18-69 year Danish adults from the general population in Copenhagen.

Explanatory variables	Atopic Dermatitis¶ % (n/n _{total})	Crude OR with 95% CI	Adjusted OR † With 95% CI	Adjusted OR † with 95% CI	Adjusted OR † with 95% CI	Adjusted OR † with 95% CI
Sex:						
Male	6.4 (91/1428)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Female	13.1 (233/1774)	2.22 (1.73-2.86)	2.41 (1.85-3.15)	2.43 (1.86-3.16)	2.39 (1.82-3.14)	2.43 (1.84-3.20)
Age (years):						
18-35	14.5 (86/593)	1 (reference), * p<0.0001	1 (reference), * p<0.001	1 (reference), * p<0.001	1 (reference), * p<0.0001	1 (reference), * p<0.0001
36-55	10.5 (163/1556)	0.69 (0.52-0.91)	0.75 (0.56-1.03)	0.75 (0.56-1.00)	0.73 (0.54-0.98)	0.72 (0.53-0.97)
56-69	7.1 (75/1053)	0.45 (0.32-0.62)	0.55 (0.39-0.78)	0.56 (0.39-0.78)	0.50 (0.35-0.72)	0.49 (0.34-0.70)
Filaggrin loss-of-function mutation: ¶¶						
No	9.1 (268/2945)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Yes	21.8 (56/257)	2.78 (2.02-3.84)	2.80 (2.00-3.92)	2.72 (1.93-3.82)	2.75 (1.94-3.89)	2.83 (2.00-4.02)
Specific IgE: ¶¶¶						
No	7.6 (184/2433)	1 (reference)	1 (reference)	1 (reference)	1 (reference)	1 (reference)
Yes	18.4 (137/746)	2.75 (2.16-3.49)	2.95 (2.30-3.78)	2.93 (2.29-3.77)	2.95 (2.28-3.81)	3.01 (2.33-3.90)
Contact sensitization to at least one allergen but not nickel and thimerosal:						
No	9.6 (295/3076)	1 (reference)	-	1 (reference)	1 (reference)	1 (reference)
Yes	23.0 (29/126)	2.81 (1.83-4.34)	-	2.44 (1.53-3.86)	2.57 (1.62-4.10)	2.53 (1.59-4.04)
Nickel sensitization:						
No	10.0 (289/2895)	1 (reference)	-	-	1 (reference)	1 (reference)
Yes	9.5 (18/190)	0.94 (0.57-1.56)	-	-	0.65 (0.38-1.10)	0.66 (0.39-1.12)
Thimerosal sensitization						
No	10.2 (317/3107)	1 (reference)	-	-	-	1 (reference)
Yes	12.5 (2/16)	1.26 (0.28-5.56)	-	-	-	1.14 (0.24-5.42)
Contact sensitization to at least one allergen						

No	9.7 (280/2886)	1 (reference)	1 (reference)	-	-	-
Yes	13.9 (44/316)	1.50 (1.07-2.12)	1.20 (0.83-1.71)	-	-	-

† = Mutually adjusted for variables shown in table.

* = p-value of trend test

OR = Odds ratio

CI = Confidence intervals

¶ = Atopic dermatitis was defined by the U.K. Working Party's diagnostic criteria for atopic dermatitis as a history of an itchy skin condition plus a minimum of two of four minor criteria ¹⁵

¶¶ = Filaggrin genotyping was performed for the loss-of-function mutations R501X and 2282del4.

¶¶¶=Analysis for IgE specific to birch, grass (timothy), cat, and mite (Dermatophagoides pteronyssinus). The analysis was judged to be positive if the measurement was in excess of 0.35 kU/l.

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