

Associations between baseline allergens and polysensitization

BERIT CHRISTINA CARLSEN, TORKIL MENNÉ AND JEANNE DUUS JOHANSEN

Department of Dermatology and Allergology, National Allergy Research Centre, Copenhagen University Hospital Gentofte, Ledreborg Allé 40, 1., 2820 Gentofte, Denmark

Background: Identification of patients at risk of developing polysensitization is not possible at present. An association between weak sensitizers and polysensitization has been hypothesized.

Objectives: To examine associations of 21 allergens in the European baseline series to polysensitization.

Patients/Methods: From a database-based study with 14 998 patients patch tested with the European baseline series between 1985 and 2005, a group of 759 (5.1%) patients were polysensitized. Odds ratios were calculated to determine the relative contribution of each allergen to polysensitization.

Results: Seven allergens – parabens mix, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine, sesquiterpene lactone mix, wool alcohols, potassium dichromate, *Myroxylon perei*ae, and cobalt chloride – showed statistically significant positive associations to polysensitization. Five allergens *p*-phenylenediamine, neomycin sulfate, epoxy resin, primin, and nickel sulfate showed statistically significant negative associations to polysensitization. For the allergens with the strongest associations, only every second individual with these particular allergies had two or more additional allergies.

Conclusions: No common denominator for the association between the allergens and the polysensitization was apparent, and any association, whether positive or negative, was relatively low. Based on these results, sensitization to specific baseline allergens cannot be used as risk indicators for polysensitization.

Key words: association; European baseline series; multiple contact allergies; polysensitization; risk factor; susceptibility. © Blackwell Munksgaard, 2008.

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Previous studies have suggested that polysensitized individuals are a unique subgroup within patients with contact allergies (1, 2). Several attempts have been made to find an indicator to identify patients at risk of developing polysensitization. Strong patch test reactions to nickel and fragrance mix are associated with having additional allergies (3), and a few genetic markers have been found to be associated with polysensitization (4, 5). Unfortunately, none of these indicators is practicable in the clinic to identify patients at risk of polysensitization.

A relatively high number of allergen associations to parabens mix and wool alcohols have been found (6). This has raised the hypothesis that

weak sensitizers in general, or parabens mix and wool alcohols as specific allergens, could be associated with polysensitization. Recently, the weak allergen parabens mix was found to be associated with polysensitization, supporting this hypothesis (7). Contrary, the strong sensitizer methyldibromoglutaronitrile was not associated with polysensitization to the same degree (7). The risk of contact allergy to neomycin sulfate also increased with additional positive reactions to other baseline allergens (8).

The aim of this study was to examine the association of 21 allergens in the European baseline series with polysensitization. Previous studies have focused on specific allergens. A definition

of polysensitization as contact allergy to three or more allergens, also referred to as multiple contact allergies, was chosen (6).

Patients and Methods

Study subjects were 14 998 patients patch tested between 1985 and 2005 at the Department of Dermatology and Allergology, Copenhagen University Hospital Gentofte, Denmark. All patients were tested with the European baseline series and, if relevant, additional allergens dependent on history. Patch test method, interpretation of patch test results, and characterization of the study population have been described previously (9).

759 patients [77.3% women and 22.7% men; median age 53.3 years, interquartile range (IQR) 26.0] with multiple contact allergies and 4419 patients (73.0% women and 27.0% men; median age 47.9 years, IQR 26.9) with one or two contact allergies were identified. The multiple-allergic group included a larger part of women (chi-squared test, $P = 0.013$), and their median age (Mann–Whitney test, $P < 0.001$) was higher than that in the single/double-allergic group.

Statistics

6.5% (977) of the patients were tested between two and five times. The last patch test performed on each individual was used as point of origin for all the statistical analyses. The statistical calculations were performed by using the SPSS software system, version 13.0 (SPSS Inc., Chicago, IL, USA).

Patients with multiple contact allergies are generally older and female compared with patients with one or two contact allergies. A simple chi squared comparison of relative sensitivity rates can, therefore, not be made. Furthermore, the probability of a positive reaction in the multiple-allergic group may not be an independent factor as the group probably has a greater likelihood of a positive response. Instead, logistic regressions with odds ratio (OR) were calculated to determine the relative contribution of each allergen to the multiple-allergic group. The ORs were calculated by logistic regression analyses with polysensitization as the dependent outcome variable and the particular allergen of interest as the independent variable as well as sex, age grouping, and interaction between sex and age grouping as covariables. Polysensitization in the logistic analyses corresponds to two or more additional positive reactions to baseline series allergens, that is excluding the positive reaction to the allergen investigated. The two baseline allergens mercaptobenzothiazole (MBT) and quaternium-15 were excluded from all

counts of additional allergies and the analyses to reduce the risk of duplicate counts of the same allergy (mercapto mix and MBT, and formaldehyde and quaternium-15).

One logistic analysis was made for each allergen. A P value < 0.05 was regarded as significant. To test if the logistic model fitted the data adequately, Hosmer's goodness-of-fit test was used.

Results

Sensitivity rates in a single/double-allergic and multiple-allergic group

Sensitivity rates for each allergen in the European baseline series for a single/double- and a multiple-allergic group are listed in Table 1 and illustrated in Fig. 1 for the multiple-allergic group. The mean number of contact allergies in the multiple-allergic and single/double-allergic group was 3.7 and 1.3 allergies per person, respectively. Sensitization to each of the 23 allergens was much more frequent in the multiple-allergic group. Nickel sulfate was the most frequent allergen in the single/double-allergic group followed by fragrance mix and *Myroxylon pereirae*, whereas fragrance mix was the most frequent allergen in the multiple-allergic group followed by nickel sulfate and *Myroxylon pereirae*. Almost every second multiple-allergic patient was allergic to fragrance mix, and 43.8% was allergic to nickel sulfate.

The distribution of allergens in the multiple-allergic group according to sensitivity rates did not show any deviating pattern. The allergens known to be frequent sensitizers were still the most frequent sensitizers, and the rare sensitizers were still comparatively rare.

Association between polysensitization and allergens in the European baseline series

The absolute frequency of one or less and two or more additional contact allergies given a specific contact allergy to 1 of 21 standard allergens is shown in Table 2.

The OR is the estimated probability or risk of being polysensitized, having two or more additional allergies, versus having one or less additional allergy in individuals with a given defined allergy to 1 of 21 standard allergens examined. ORs were calculated in two different populations, a population of patch-tested individuals (Table 3) and a population of individuals with at least one contact allergy (Table 4).

In the patch-tested population, parabens mix, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine (IPPD), wool alcohols, and sesquiterpene lactone mix (SL mix) had the greatest risk of being part of a complex of multiple contact allergies (OR 7.2,

Table 1. Positive reactions, cases tested, and absolute frequencies calculated for each allergen in the European baseline series for a single/double-allergic and a multiple-allergic group

Allergens	Single/double contact allergies (<i>n</i> = 4419)		Multiple contact allergies (<i>n</i> = 759)	
	Positive reactions (cases tested)	Absolute frequency (%)	Positive reactions (cases tested)	Absolute frequency (%)
Fragrance mix I	788 (4410)	17.9	369 (755)	48.9
Nickel sulfate	1459 (4376)	33.3	326 (744)	43.8
<i>Myroxylon perei</i>	391 (4416)	8.9	267 (758)	35.2
Colophonium	365 (4414)	8.3	218 (758)	28.8
Cobalt chloride	346 (4416)	7.8	198 (756)	26.2
Formaldehyde	265 (4415)	6.0	163 (756)	21.6
Potassium dichromate	206 (4415)	4.7	157 (756)	20.8
Thiuram mix	263 (4415)	6.0	140 (758)	18.5
MCI/MI	157 (4387)	3.6	107 (748)	14.3
Neomycin sulfate	313 (4414)	7.1	107 (756)	14.2
PPD	224 (4409)	5.1	95 (756)	12.6
SL mix	82 (3869)	2.1	77 (656)	11.7
Quaternium-15	58 (4418)	1.3	73 (757)	9.6
Wool alcohols	70 (4418)	1.6	66 (759)	8.7
Mercapto mix	38 (4415)	0.9	62 (758)	8.2
PTBFR	124 (4417)	2.8	58 (759)	7.6
MBT	24 (4378)	0.5	54 (744)	7.3
Primin	115 (4417)	2.6	53 (755)	7.0
Clioquinol	63 (4418)	1.4	47 (759)	6.2
Epoxy resin	123 (4413)	2.8	43 (758)	5.7
Parabens mix	42 (4417)	1.0	41 (759)	5.4
IPPD	40 (4410)	0.9	39 (756)	5.2
Benzocaine	53 (4419)	1.2	29 (758)	3.8
Total	5609		2789	

IPPD, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine; MBT, mercaptobenzothiazole; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; PPD, *p*-phenylenediamine; PTBFR, *p*-tertiary butylphenol formaldehyde resin; SL mix, sesquiterpene lactone mix.

7.1, 6.5, and 6.3, respectively). Primin had the lowest association to polysensitization (OR 2.3). There was no clear-cut point that divided the allergens into groups. All standard allergens examined were significantly and positively associated to polysensitization.

In the population of contact allergic individuals, SL mix, parabens mix, IPPD, and wool alcohols still had the greatest risk of being part of a complex of multiple contact allergies; however, the ORs

were a lot smaller (OR 1.7, 1.7, 1.6, and 1.5, respectively). The bottom nine allergens showed a negative association to polysensitization. Only 12 allergens were statistically significant associated, either positively or negatively, to polysensitization.

Discussion

Two different populations were used for the logistic regression analyses, a population of

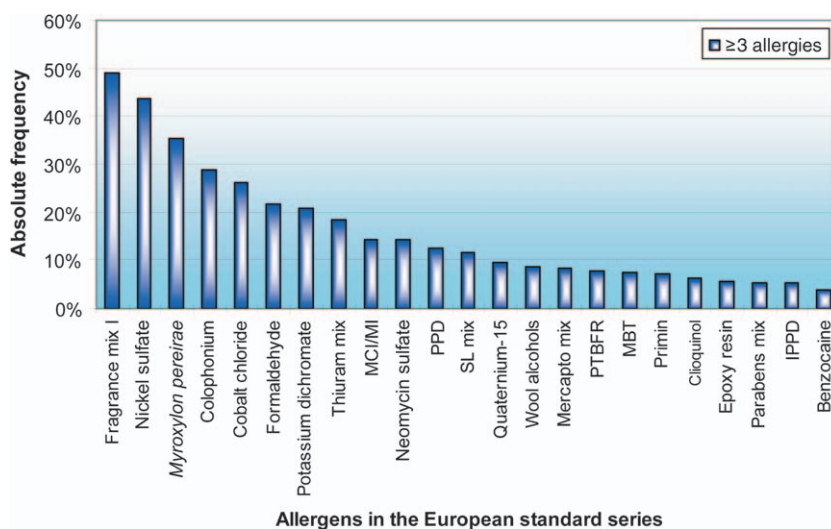


Fig. 1. Allergen sensitivity rates among patients with multiple contact allergies (*n* = 759). IPPD, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine; MBT, mercaptobenzothiazole; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; PPD, *p*-phenylenediamine; PTBFR, *p*-tertiary butylphenol formaldehyde resin; SL mix, sesquiterpene lactone mix.

Table 2. Frequency of one or less and two or more additional contact allergies given a specific contact allergy to 1 of 21 baseline allergens^a

Standard allergens (n)	One or less additional contact allergies, n (%)	Two or more additional contact allergies, n (%)
Parabens mix (83)	42 (50.6)	41 (49.4)
SL mix (159)	82 (51.6)	77 (48.4)
Wool alcohols (136)	72 (52.9)	64 (47.1)
IPPD (79)	42 (53.2)	37 (46.8)
Mercapto mix (100)	55 (55.0)	45 (45.0)
Potassium dichromate (363)	208 (57.3)	155 (42.7)
Clioquinol (110)	63 (57.3)	47 (42.7)
<i>Myroxylon pereirae</i> (658)	392 (59.6)	266 (40.4)
MCI/MI (264)	163 (61.7)	101 (38.3)
Colophonium (583)	368 (63.1)	215 (36.9)
Cobalt chloride (544)	350 (64.3)	194 (35.7)
Benzocaine (82)	53 (64.6)	29 (35.4)
Thiuram mix (403)	273 (67.7)	130 (32.3)
PTBFR (182)	124 (68.1)	58 (31.9)
Fragrance mix I (1157)	798 (69.0)	359 (31.0)
Formaldehyde (428)	297 (69.4)	131 (30.6)
Primin (168)	120 (71.4)	48 (28.6)
PPD (319)	228 (71.5)	91 (28.5)
Epoxy resin (166)	123 (74.1)	43 (25.9)
Neomycin sulfate (420)	316 (75.2)	104 (24.8)
Nickel sulfate (1785)	1472 (82.5)	313 (17.5)

IPPD, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; n, individuals with positive reaction; PPD, *p*-phenylenediamine; PTBFR, *p*-tertiary butylphenol formaldehyde resin; SL mix, sesquiterpene lactone mix.

^aMercaptobenzothiazole and quaternium-15 was excluded from count of additional allergies.

patch-tested individuals and a population of contact allergic individuals. A population of patch-tested individuals was used to be able to compare results with formerly published data. The outcome, based on this patch-tested population, represents the risk of polysensitization given a specific contact allergy compared with the risk of polysensitization if you do not have this allergy including individuals with no contact allergy at all. The analyses showed that all allergens, to varying extent, were associated to polysensitization; this can be explained by the choice of reference group. The largest part of the reference group consisted of individuals without any contact allergy at all. The risk of polysensitization, given a defined contact allergy, is obviously greater than the risk of polysensitization when not having a contact allergy at all. The relative distribution of allergens according to ORs represents the true differences between allergens. Parabens mix had the strongest association to polysensitization with an OR of 7.2, correlating well with a previously found OR of 7.4 (7).

The outcome of the logistic regression analyses based on a population of patch-tested individuals is susceptible to selection bias. Increasing the num-

Table 3. Risk of being polysensitized (having two or more additional contact allergies) versus having zero or one additional contact allergy in individuals with a given defined allergy to 1 of 21 allergens in the European baseline series, illustrated by odds ratios^a

Allergens*	n	Odds ratio	95% confidence interval
Parabens mix	14 996	7.2	4.6–11.2
IPPD	14 964	7.1	4.5–11.1
Wool alcohols	14 994	6.5	4.6–9.1
SL mix	13 198	6.3	4.6–8.7
Mercapto mix	14 989	6.0	4.0–9.0
<i>Myroxylon pereirae</i>	14 988	5.8	4.9–6.8
Potassium dichromate	14 979	5.8	4.7–7.2
Clioquinol	14 996	5.5	3.7–8.1
Cobalt chloride	14 976	4.9	4.1–6.0
Colophonium	14 985	4.8	4.0–5.7
Fragrance mix I	14 971	4.5	3.9–5.2
MCI/MI	14 878	4.4	3.4–5.7
Thiuram mix	14 982	3.5	2.8–4.4
Benzocaine	14 995	3.5	2.2–5.6
Formaldehyde	14 980	3.3	2.6–4.0
PTBFR	14 994	3.2	2.3–4.4
PPD	14 966	3.0	2.3–3.8
Epoxy resin	14 987	2.6	1.8–3.7
Nickel sulfate	14 845	2.5	2.2–2.9
Neomycin sulfate	14 978	2.4	1.9–3.0
Primin	14 986	2.3	1.7–3.3

IPPD, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; n, total number of individuals patch tested; PPD, *p*-phenylenediamine; PTBFR, *p*-tertiary butylphenol formaldehyde resin; SL mix, sesquiterpene lactone mix.

*For all allergens $P < 0.05$, statistically significant association.

^aThe analyses were adjusted for sex, age, and interaction between sex and age.

ber of individuals selected for patch testing will result in increasing ORs. Therefore, we made logistic regression analyses for a population of contact allergic individuals. The number of individuals with contact allergies is primarily determined by exposure (10–14). Although the number of contact allergic individuals will increase with increasing number of individuals patch tested, both the sensitization rate to the particular allergen of interest and the reference group will increase, not influencing the ORs to the same degree.

The outcome based on a contact allergic population represents the risk of polysensitization given a specific contact allergy compared with the risk of polysensitization in general among a contact allergic group. The risk of being polysensitized in this population, given a defined contact allergy, is a lot lower than that in the former population; however, the ranking of allergens according to ORs do not switch markedly. The same allergens account for the top sensitizers with the strongest association. Interestingly, the bottom allergens have negative associations to polysensitization. The outcomes, based on the contact

Table 4. Risk of being polysensitized (having two or more additional contact allergies) versus having zero or one additional contact allergies in individuals with a given defined allergy to 1 of 21 allergens in the European baseline series, illustrated by odds ratios^a

Allergens	<i>n</i>	Odds ratio	95% confidence interval
SL mix*	4509	1.7	1.2–2.3
Parabens mix*	5158	1.7	1.1–2.6
IPPD*	5148	1.6	1.05–2.6
Wool alcohols*	5159	1.5	1.1–2.1
Mercapto mix	5155	1.5	1.0–2.2
<i>Myroxylon pereirae</i> *	5156	1.4	1.2–1.7
Potassium dichromate*	5153	1.4	1.2–1.8
Cobalt chloride*	5154	1.3	1.1–1.6
Clioquinol	5159	1.3	0.9–1.9
Colophonium	5154	1.2	1.0–1.4
MCI/MI	5117	1.2	0.9–1.5
Fragrance mix I	5147	1.1	0.9–1.2
Thiuram mix	5155	0.9	0.7–1.1
Benzocaine	5159	0.9	0.6–1.5
Formaldehyde	5154	0.8	0.7–1.0
PPD*	5147	0.8	0.6–0.97
PTBFR	5158	0.8	0.6–1.2
Neomycin sulfate*	5152	0.6	0.4–0.7
Epoxy resin*	5153	0.6	0.5–0.9
Primin*	5154	0.6	0.4–0.9
Nickel sulfate*	5102	0.5	0.4–0.6

IPPD, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; *n*, total number of contact allergic individuals; PPD, *p*-phenylenediamine; PTBFR, *p*-tertiary butylphenol formaldehyde resin; SL mix, sesquiterpene lactone mix.

**P* < 0.05, statistically significant association.

^aThe analyses were adjusted for sex, age, and interaction between sex and age.

allergic population, are point of reference for the rest of the discussion.

Seven allergens showed statistically significant positive associations to polysensitization. Individuals sensitized to SL mix, parabens mix, IPPD, wool alcohols, *Myroxylon pereirae*, potassium dichromate, and cobalt chloride carry a greater risk or are more likely to be polysensitized compared with the general risk of being polysensitized in a contact allergic group. Of these, only parabens mix has previously been shown to be associated to polysensitization (7). Mercapto mix did not reach statistical significance in our study but ranks as the fifth strongest association. Mercapto mix has previously been shown to occur more frequently in association with other allergens than as a single allergy (15). Five allergens turned out to have statistically significant negative associations to polysensitization; *p*-phenylenediamine (PPD), neomycin sulfate, epoxy resin, primin, and nickel sulfate. Former studies have also shown that nickel sulfate is not associated with polysensitization (15, 16).

The top four allergens with the highest ORs are all allergens with relatively low sensitivity rates (parabens mix, IPPD, wool alcohols, and SL mix) (9). Although these uncommon sensitizers show a strong association to polysensitization, it does not mean that the majority of polysensitized individuals acquire allergies to uncommon sensitizers, hence the absolute sensitivity rates. Ubiquitous and potent allergens were the most frequent sensitizers, and the non-ubiquitous and weak allergens were uncommon sensitizers among multiple-allergic individuals, as would be expected because of exposure and allergen potency. If a particular allergen had stood out in the multiple-allergic group, it could have been interpreted as this allergen predisposed to polysensitization. Because this is not the case, environmental exposure seems to be the primary determinant of which patterns of allergens we see in polysensitization.

Increased risk of polysensitization can be explained theoretically by an inherent increased susceptibility; intense, frequent and/or persistent exposure; or acquired susceptibility because of barrier disruption and inflammation.

Polysensitized individuals with allergies to more ubiquitous allergens and who do not have a history of any unusual exposures have been shown to have an increased inherent susceptibility towards development of contact allergy (1). The increased susceptibility was not caused by an upregulated state in the immune system because of the acquisition of allergies or the inflammation but was an inherent property. In low exposure situations, the inherent susceptibility may be the decisive factor why only a small proportion of individuals exposed develop contact allergy and multiple contact allergies.

The development of polysensitization can also be explained by a gross, frequent, or persistent exposure. Several exposure factors increases the risk of sensitization; allergen dose and potency (17, 18); occlusion, extent and duration of exposure (11, 12); and simultaneous exposure to other allergens or irritants (13, 19–21). Under extreme conditions, anyone exposed can become sensitized regardless of any inherent susceptibility (11, 22). In everyday life, occupation and treatments for skin diseases, for example leg dermatitis, are examples of settings with potentially gross exposures, often to several allergens, increasing the risk of polysensitization.

Damaged skin can be considered as an acquired susceptibility. The presence of inflammation or disruption of skin barrier increases the likelihood for induction of contact allergies (11). Damaged skin may be maintained by persistent exposure to allergens or irritants or because of other skin

diseases or because of genetic predisposition to a disrupted skin barrier. Recently, loss-of-function mutations in the profilaggrin gene have been associated to a defect skin barrier (23) and to nickel contact allergy (24).

A possible interpretation of the results showing positive and negative associations with polysensitization is the presence or absence of associated exposure with other allergens in the baseline series. The allergens with significant positive associations often occur in exposure settings with multiple allergen exposure, and the allergens with significant negative associations are often found in isolated exposure settings. However, some allergens without statistically significant associations with polysensitization also occur in settings with multiple allergen exposure or alone.

Within the two groups of allergens with, respectively, positive and negative associations, there is no common denominator. Some of the allergens are ubiquitous, others are not, and some of the allergens are weak sensitizers, for example parabens mix and wool alcohols, and others are strong sensitizers, for example IPPD. Some allergens are typically associated to exposure settings with high intensity, for example parabens mix and wool alcohols in leg ulcer treatment and epoxy resin and IPPD as occupational allergens, and others are not, for example potassium dichromate and nickel sulfate.

Polysensitization may also occur in the context of cross-reactivity. However, cross-reactivity does not seem to explain the ranking of allergens. Allergens known to cross-react display opposite signs in their association with polysensitization. For example, IPPD is positively associated, whereas PPD is negatively associated, and benzocaine is not associated at all.

The allergens with, respectively, positive and negative associations with polysensitization have highly variable chemical structures, allergen potency, and typical exposure settings. No pattern within the negatively or positively associated group of allergens is obvious. Between the two groups is a general appearance of presence or absence of associated exposure, which is also true for the allergens without any significant association. This interpretation can explain the distribution of allergens according to ORs but do not provide a risk indicator for polysensitization.

Based on these results, sensitization to specific allergens cannot be used as risk indicators for polysensitization. Because of multiple testing, the results need to be confirmed in other studies. For all the allergens, the effect size of an association, whether positive or negative, is relatively low. For the allergens with the strongest associations, only

about every second individual with these particular allergies have two or more additional allergies. Finally, it is not known in what order the allergies were acquired. If the specific sensitization of interest was acquired as the third, fourth, or fifth allergy, it cannot be used as an indicator.

There is a lack of a common explanatory denominator for the association between the allergens and the polysensitization. The individuals in the contact allergic population have already displayed an ability to acquire contact allergies, and likely, this ability increases the risk of polysensitization but not the specific allergens themselves.

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Address:

Berit Christina Carlsen, MD
Department of Dermatology and Allergology
National Allergy Research Centre
Copenhagen University Hospital Gentofte
Ledreborg Allé 40, 1.
2820 Gentofte
Denmark
Tel: +45 3977 7340
Fax: +45 3977 7118
e-mail: bccarlsen@dadlnet.dk