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PATIENTS WITH MULTIPLE CONTACT ALLERGIES: POPULATION CHARACTERISTICS AND CLINICAL PRESENTATION

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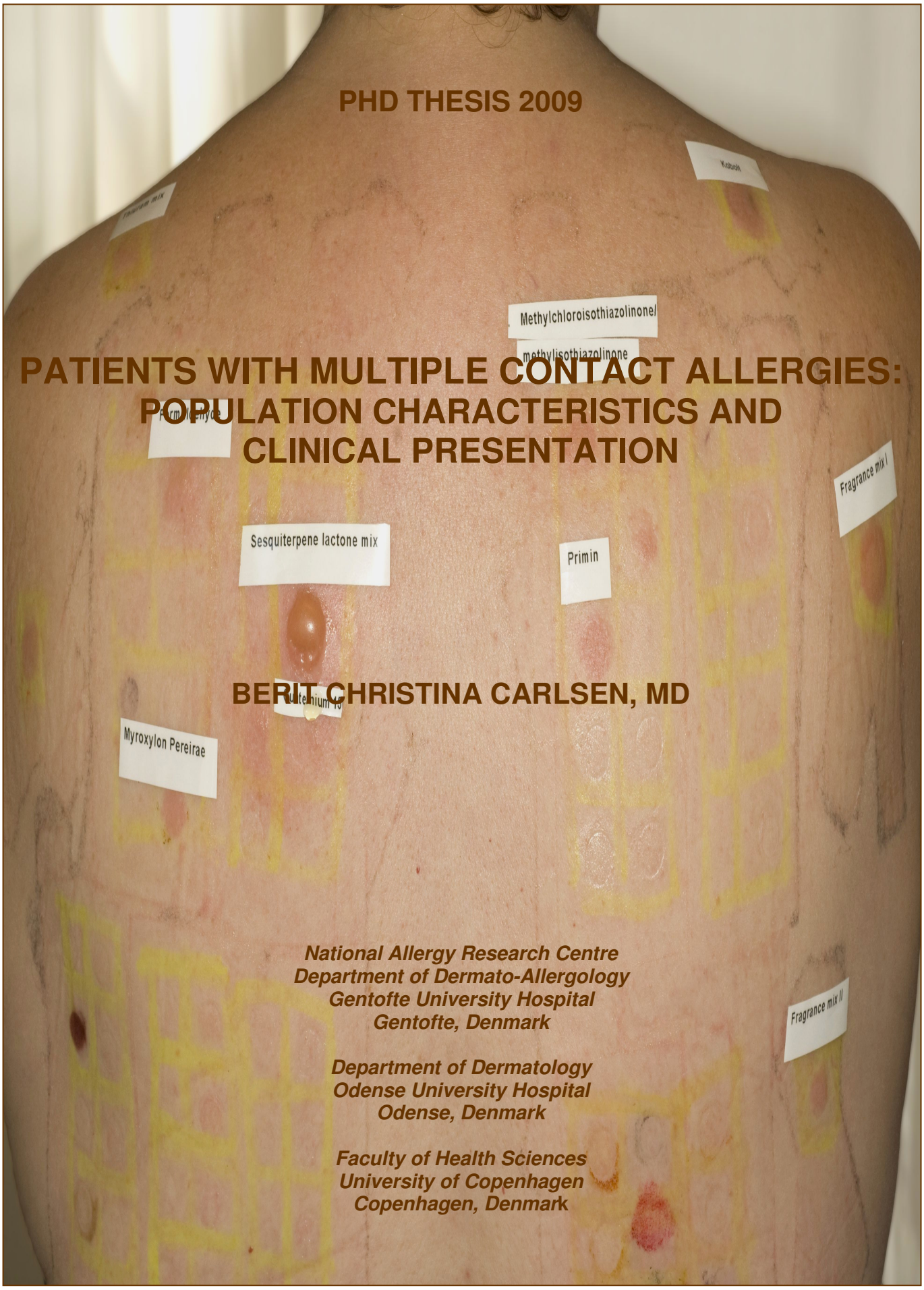

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”Alene deltagelsen i denne undersøgelse og de erindringer det har medført, har fremkaldt kløe over hele kroppen...”

(The participation in this survey alone and the recollections it has brought to mind, have led to an itching sensation over my entire body...)

anonymous questionnaire participant I

”man tænker meget over hvad man ikke kan uden hænder…”

(you think a lot about what you cannot do without hands...)

anonymous questionnaire participant II

This PhD thesis is based on the following 5 manuscripts:

Study part I: The database study

I. **Carlsen BC**, Menné T, Johansen JD. Twenty Years of Standard Patch Testing in an Eczema Population with focus on patients with multiple contact allergies. Contact Dermatitis. 2007 Aug; 57(2): 76-83.
II. **Carlsen BC**, Menné T, Johansen JD. Associations between baseline allergens and polysensitization. Contact Dermatitis. 2008 Aug; 59(2): 96-102.

Study part II: The dose-response study

III. **Carlsen BC**, Fischer LA, Sosted H, Voelund A, Menné T, Johansen JD. Patch test dose-response study: polysensitized individuals do not express lower elicitation thresholds than single/double-sensitized individuals. Br J Dermatol. 2009 Jan; 160(1): 103-6.

Study part III: The questionnaire study

IV. **Carlsen BC**, Andersen KE, Menné T, Johansen JD. Characterization of the polysensitized patient: a matched case-control study. Contact Dermatitis. 2009 Jul; 61 (1): 22-30.
V. **Carlsen BC**, Andersen KE, Menné T, Johansen JD. Sites of dermatitis in a patch test population: hand dermatitis is associated with polysensitization. Br J Dermatol. 2009 May 5. [Epub ahead of print].

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PREFACE

Contact allergy is a cell-mediated immune reaction, primarily caused by environmental exposure to low-molecular weight chemical substances. Not all individuals who are exposed will develop contact allergies, and it is even less likely that they will develop multiple contact allergies. Patients with multiple contact allergies have received limited attention in research studies. This PhD thesis focuses on patients with multiple contact allergies.

The work was conducted during a 3 year engagement from June 2006 to June 2009 at the National Allergy Research Centre, Department of Dermato-Allergology, Gentofte University Hospital under the excellent guidance of Professor Jeanne Duus Johansen, Professor Torkil Menné and Professor Klaus Ejner Andersen. Secretarial assistance, statistical assistance and IT support were kindly provided from Susanne Schweitz, Aage Vølund and Søren Gade, respectively. The work was supported by grants from the Danish National Board of Health, the Danish Environmental Protection Agency and the Royal Court Furrier Aage Bang´s Foundation. The PhD thesis is based on the results from 3 research studies: 2 epidemiological studies and 1 experimental study, presented in 5 manuscripts (see previous page).

I wish everybody happy reading….

Berit Carlsen, MD
May 2009

ABBREVIATIONS

AE	Atopic eczema
APL	A Programming Language
CI	Confidence Interval
D	Day
DNCB	Dinitrochlorobenzene
EBS	European Baseline Series
IPPD	*N*-Isopropyl-*N*-phenyl-*p*-phenylenediamine
IR	Irritant reaction
IQR	Interquartile range
MBT	Mercaptobenzothiazole
MCI/MI	Methylchloroisothiazolinone/methylisothiazolinone
MDBGN	Methyldibromo glutaronitrile
NOSQ	Nordic Occupational Skin Questionnaire
NT	Not Tested
NT:S	Not Tested: Sensitized
OR	Odds ratio
PPD	*p*-phenylenediamine
PTBFR	*p*-tertiary butylphenol formaldehyde resin
SL mix	Sesquiterpene lactone mix
SPSS	Statistical Package for the Social Sciences
STSC	Scientific Time Sharing Corporation
UK	United Kingdom
UV	Ultraviolet

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1. BACKGROUND

1.1. Defining polysensitization

Throughout this PhD thesis, polysensitization is used synonymously with multiple contact allergies and polysensitization / multiple contact allergies is defined as 3 or more contact allergies. No gold standard exists for the definition of polysensitization. Some authors use 2 or more, others 3 or more contact allergies [1;2]. Recently, a definition of polysensitization as 3 or more contact allergies has been recommended [3].

1.2. Epidemiology

The prevalence of patients with multiple contact allergies ranges from 0.7% in one general population to 4-7% in hospital patch test populations [1;4-6]. In comparison, the overall prevalence of contact allergies ranges from 15% in general populations to 28-37% in hospital patch test populations [1;4-6]. The prevalence of multiple contact allergies in dermatology private practices is unknown.

Polysensitized individuals are mainly described in case reports, excluding reports on polysensitization and patients with stasis dermatitis and leg ulcers. Most of the reports can be placed in one of four groups: patients exposed to topical drugs other than leg ulcer patients [7-9], patients with leg ulcers and exposures related to wound treatment [10-13], patients with occupational exposure [14-16], and patients with allergies to ubiquitous allergens and with low-risk exposure e.g. consumer exposure [17-19].

Case reports do not reveal the true composition of patients with multiple contact allergies. It is unknown what the relative proportion is of patients with multiple contact allergies caused by occupational exposure, by topical treatment regimes, or where no extraordinary exposure is identified. Sites of dermatitis in patients with multiple contact allergies are also unknown. Patients with stasis dermatitis and leg ulcers often develop multiple contact allergies [10], but a large fraction of polysensitized individuals may have dermatitis in other skin areas. In patients selected because of a fragrance allergy, hand dermatitis has been reported to occur less frequently in polysensitized (26.0%) than in monosensitized patients (44.4%) [20]. Demographic characteristics such as sex, age, and socioeconomic status are largely unknown. A larger fraction of patients above 40 years of age has 2 or more contact allergies compared with patients below 40 years of age [4].

Only weak indications of disease duration, course and severity exist. Patients with multiple contact allergies are mainly found in hospital patch test populations compared with general populations. This observation might relate to chronic or recurrent dermatitis either caused by multiple eliciting environmental allergens and repeated exposures, or difficulty in identifying or avoiding eliciting allergens. Chronic or recurrent dermatitis leads to a need for medical attention, repeated dermatology visits and referral to hospital departments. Patients with multiple contact allergies also demonstrate an increased elicitation response compared with monosensitized and healthy controls [1] when experimentally sensitized and challenged with dinitrochlorobenzene (DNCB). Such increased reactivity to allergen exposure may result in more severe dermatitis.

One study showed an increased occurrence of generalized dermatitis in polysensitized patients (39%) compared with monosensitized patients (25.5%) [20].

Persistent allergen exposure will maintain the clinical response. It is a logical assumption that the high number of contact allergies per individual in polysensitized individuals contributes to the complexity of avoiding all allergens resulting in persistent disease. One study showed an inverse correlation between number of contact allergies and improvement [21]. Another study could not find any difference between patients with multiple contact allergies and patients with one allergy with regard to dermatitis outbreak frequency or severity [22]. A 30-year old paper showed that a larger fraction of patients who seek permanent disability pension had multiple contact allergies compared with a reference hospital cohort [23]. Multiple allergies seemed to lead to an unfavourable outcome, but several factors other than disease severity, e.g. social conditions, can influence who seeks disability pension. The outcome of the study would most likely be different if it were re-examined today.

1.3. Allergens in combination

Multiple contact allergies may occur in the context of cross-reactivity, associated exposure, angry back phenomenon, exited skin syndrome or as true random coincidences of multiple positives to structurally and environmental unrelated allergens.

Cross-reactivity occurs between two allergens with chemically related structures, when one allergen is metabolized to or releases a compound identical or similar to another allergen, or when two allergens produce identical / similar metabolites [24].

Concomitantly occurring allergies because of associated exposure refers to allergens that frequently occur together in the same products or in the same environment but do not have similar chemical structures.

Angry back and excited skin syndrome are phenomena that can be considered as sources of errors in simultaneously appearing positive patch test reactions. In the angry back syndrome, strong positive reactions heighten reactivity at nearby patch tests, creating false positive reactions [25;26]. In the excited skin syndrome, such hyperreactivity involves the entire skin and not only the back [27]. Distant dermatitis is assumed to cause generalised skin hyperirritability; consequently, strong positive reactions create heighten reactivity not only at nearby patch test sites but also at more distant patch test sites [27]. The angry back and excited skin syndrome should be distinguished from instances where dermatitis appear over the entire patch test area and are not confined to the patch test chambers which were not included in the original description [25;28].

Combinations of allergens can occur at higher frequencies than predicted from single sensitivities. The majority of studies that have tried to identify such clusters of allergens have focused on allergens from the standard series. Varying numbers of statistically significant associated duplet allergen combinations have been identified ranging from 13 to 166 combinations [29-33]. Only very few triplet combinations have been identified and no combinations larger than triplet have been reported. Some allergen clusters are caused by cross-reactivity, others by associ-

ated exposure, and some combinations are not explained by these mechanisms but are true random but significant coincidences. Triplet clusters have been identified for nickel / cobalt / chrome [5;34], fragrance mix / colophony / compositae mix [35], fragrance mix / colophony / Myroxolon pereirae [36], thiuram mix / mercapto mix / carba mix [4], *p*-phenylenediamine (PPD) / nickel / benzocaine [4] and PPD / *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine (IPPD) / benzocaine [37].

The tendency of a particular allergen to occur in combination or isolated varies [4]. Nickel tends to occur alone, even though it is often the most frequent allergen found; mercapto mix tends to occur in combination with other allergens [4]. Allergy to parabens mix is associated with polysensitization [38] in contrast to allergy to methyldibromo glutaronitrile, which is not associated with polysensitization to the same degree [38]. The risk of contact allergy to neomycin sulphate increases with additional positive reactions to other standard allergens [39]. Compositae mix, fragrance mix, Myroxylon pereirae and formaldehyde allergy is often seen in combination with additional allergies [20;22;40]. Whether a particular allergen or subgroup of allergens triggers a cascade of secondary sensitizations is unknown. The order of development of contact allergies for patients with multiple contact allergies has not been reported.

1.4. Evidence of polysensitization as a phenotype for increased susceptibility

Environmental exposure is an absolute requirement for sensitization [41;42], but it is not the sole driver [43;44]. Several genetic markers have been positively associated with contact allergy and with polysensitization [2;45-52]. The question arises whether polysensitized patients are especially susceptible to development of contact allergies.

The predicted prevalence can be calculated from single sensitivity rates but requires that two assumptions are made: 1. Combinations of allergens are not inherently connected in any way, and 2. Individuals are not inherently different in susceptibility to development of contact allergies. Polysensitized patients appear more frequently than would be expected by chance, both in a dermatitis population [1] and in the general population [6].

Patients with 3 or more contact allergies develop more new positives than patients with 1-2 contact allergies when tested additional times [53]. It may reflect an increased susceptibility to development of contact allergies, or be confounded by gross and frequent exposure or by chronic disease with disrupted skin barrier and inflammation caused by the acquisition of several allergies.

Patients with multiple contact allergies show functional changes in the induction and elicitation response when experimentally exposed to DNCB in comparison with mono-allergic patients and healthy controls [1]. Patients with multiple contact allergies were sensitized to DNCB with lower induction doses. The amplification of response to increasing sensitizing dose was greater and the observed skin reaction at challenge was more pronounced. Mono-allergic individuals expressed intermediate values and within the group of multiple-allergic patients, the reaction to DNCB increased with rarity of the combinations of contact allergies, implying a graded susceptibility. The increased susceptibility was a feature of only the subgroup of patients with multiple

contact allergies caused by ubiquitous baseline exposures [1;42], not for multiple-allergic patients with leg ulcers or with an occupational pathogenesis. In another study, leg ulcer patients were not more likely to be sensitized than was a control group [54]. The reported increased susceptibility was not a consequence of inflammation since all patients were dermatitis-free at the time of testing. Whether the increased susceptibility and reactivity also adhere to irritants has not been studied. The study has not been replicated with other allergens.

1.5. Risk factors for polysensitization

Patients with leg ulcers are undoubtedly at risk for polysensitization: 73-80% of leg ulcer patients are sensitized and 53-57% are polysensitized [10-13]. The risk correlates with duration of disease [11-13]. Whether specific high risk occupations, specific treatments other than wound treatment or specific skin sites of dermatitis constitute a high risk for polysensitization is plausible but not documented.

Two polymorphisms in the genes for cytokines interleukin-16 and tumour necrosis factor-α are associated with polysensitization [49;50] but are also associated with dermatitis of other causes [50;55;56]. Rapid acetylator N-acetyltransferase alleles [45;46], glutathione-S-transferase polymorphisms [47;48] and filaggrin mutations [2;52] have been associated with contact allergy but have not been examined in relation to polysensitization.

Positive patch test reactions to parabens mix is associated with polysensitization [38]. Parabens mix might act as confounder for a specific exposure.

Strong patch test reactions increase the likelihood of additional positive reactions and are also associated with strong reactivity to the additional contact allergens [29;57]. Strong patch test reactions can represent a general, increased reactivity or a high environmental exposure load, increasing the likelihood of strong patch test reactions and additional allergies.

2. AIMS OF THE STUDIES

The overall aim of this PhD thesis was to contribute to a better characterization of patients with multiple contact allergies with regard to 1) the population demographics and composition, and 2) the clinical presentation; and to examine 3) the elicitation response when exposed to allergens.

The specific aim of each study part and manuscript was as follows:

Study part I – the database study

I. To identify polysensitized patients within a Danish patch test population and determine prevalence and population demographics.

II. To determine allergen-specific sensitivity rates in patients with polysensitization and examine the association of 21 allergens in the European Baseline Series with polysensitization.

Study part II – the dose-response study

III. To investigate the elicitation dose-response profile in polysensitized patients compared with a reference group of single/double-sensitized patients for the allergens nickel sulphate, methyldibromo glutaronitrile and *p*-phenylenediamine.

Study part III – the questionnaire study

IV. To examine the occurrence, duration and course of dermatitis in polysensitized patients and to examine potential risk factors for polysensitization, including the association between polysensitization and atopic eczema.

V. To determine the distribution of dermatitis on the skin at time of debut and to examine the extent of dermatitis, also at time of debut, in polysensitized patients.

3. MATERIALS AND METHODS

3.1. STUDY PART I, MANUSCRIPT I & II

3.1.1. Database

Results from all patch tests performed at the Department of Dermato-Allergology, Gentofte University Hospital, Denmark are registered in a database in the electronic network of the Capital Region of Denmark. The data are entered manually into the database and the original data sheets are stored. Six-monthly reports are generated on chosen parameters and any inconsistencies checked. Annual reports are made to ascertain that all patch tested individuals have been entered into the database. Access to the database is restricted to a few individuals with mandatory user authentication.

3.1.2. Study population

The study population consisted of all patients patch tested with the European Baseline Series during the years 1985-2005. Only study subjects tested with at least 16 of the 23 allergens in the European Baseline Series were included; 14,998 study subjects were patch tested during these 20 years with 16,108 patch test events registered; 977 study subjects were tested between 2-5 times. Only 3 of 14,998 individuals were tested with the minimum of 16 allergens.

3.1.3. European Baseline Series

The European Baseline Series (EBS) consisted of 23 allergens at launching of the database study (TABLE 1). During 1985-2005, 15 allergens did not change concentration or composition, 3 allergens changed concentration, and 4 allergens changed composition (TABLE 1). The sesquiterpene lactone mix (SL mix) was unavailable for testing from 1985 to May 27th 1987. Different allergen concentrations and compositions used were not evaluated separately.

3.1.4. Patch test method and readings

Throughout the entire period, patch testing was done with Finn Chambers®, Scanpor Tape® and TROLAB® patch test allergens applied to the upper back. The occlusion time was 48 hours and readings were done on Day (D) 2, D3/4 and D7 according to the recommendation from the International Contact Dermatitis Research Group [58]. Homogeneous redness and infiltration in the entire test area was scored as a 1+ reaction. Homogeneous redness, infiltration, and vesicles in the test area was scored as a 2+ reaction, and homogeneous redness, infiltration, and coalescing vesicles in the test area was scored as a 3+ reaction. A 1+, 2+ and 3+ reaction was interpreted as a positive response. Irritant reactions, doubtful (+?) and negative reactions were interpreted as a negative response. Some study subjects were not tested with the complete set of allergens either because of known sensitivity based on previous testing, registered as “Not Tested: Sensitized” (NT:S) or for unknown reasons, registered as “Not Tested” (NT). NT-readings were categorized as missing data. NT:S-readings were categorized as positive responses.

3.1.5. Data validation

Data from the database were exported by an authorized operator. Each variable was checked for outliers and missing data by frequency tables. Internal consistencies were checked by cross-

TABLE 1: *Allergens in the European Baseline Series 2005*

ALLERGENS IN THE EUROPEAN BASELINE SERIES 1985-2005	CONCENTRATION and VEHICLE
Potassium dichromate	0.50% petrolatum
Neomycin sulphate	20.0% petrolatum
Thiuram mix	1.0% petrolatum
Phenylenediamine, para- *1985-1988 Phenylenediamine, para-*	1.0% petrolatum *0.5% petrolatum*
Cobalt chloride	1.0% petrolatum
Benzocaine	5.0% petrolatum
Formaldehyde	1.0% aqua
Colophony *1985-1986 Colophony*	20.0% petrolatum *60.0% petrolatum*
Clioquinol *1985-1994 Quinoline mix*	5.0% petrolatum *6.0% petrolatum*
Isopropyl-N-phenyl PPD, N- (IPPD) *1985-1993 Black rubber mix*	0.10% petrolatum *0.6% petrolatum*
Wool Alcohols	30.0% petrolatum
Mercapto mix *1985-1995 Mercapto mix*	1.0% petrolatum *2.0% petrolatum*
Epoxy resin	1.0% petrolatum
Parabens mix *1985-1995 Parabens mix*	16.0% petrolatum *15.0% petrolatum*
Tert.-but.phenolformaldehyde (BPF) resin, para-	1.0% petrolatum
Fragrance mix I with Sorbitan Sesquioleate (5%) *1985-1992 Fragrance mix I*	8.0% petrolatum *8.0% petrolatum*
Myroxolon pereirae	25.0% petrolatum
Sesquiterpene lactone mix *1985-1987 SL mix not invented*	0.10% petrolatum
Quaternium-15	1.0% petrolatum
Nickel sulphate	5.0% petrolatum
(Cl)Me-isothiazolinone	0.01% aqua
Mercaptobenzothiazole (MBT)	2.0% petrolatum
Primin	0.01% petrolatum

Changes in composition and/or concentration during 1985-2005 are printed in *italics*.

tabulations where possible. Inconsistencies between test results in study subjects tested multiple times were also checked by cross-tabulations. Missing data, outliers, and inconsistencies were all checked against the original data. All “NT” and “NT:S” registrations were counter-checked in the original data sheets to verify the correctness of registration.

Missing data occurred only for the allergens IPPD and SL mix (TABLE 2). The large number of missing data for SL mix corresponds to the study subjects who were not tested with SL mix January 1st 1985 - May 27th 1987 and September 23rd 1987 - November 17th 1987 where the SL mix was not available for testing. There were few NT and NT:S registrations (TABLE 2); 12,926 patients (86.2%) were patch tested with the entire EBS (23 allergens) or had a known positive reaction (NT:S). Excluding the missing data because of unavailability of SL mix in the early years, 95.5% of patients were tested with all allergens in the EBS available at the time of testing or had a known positive reaction diagnosed by previous patch tests (NT:S).

3.1.6. Data analyses

The statistical analyses were performed in the statistical software system SPSS® version 13.0 (SPSS Inc., Chicago, Illinois, USA). 6.5% of patients were tested 2-5 times. Overall and allergen-specific prevalence rates, nominal regression analysis in manuscript I and all data and statistical analyses in manuscript II were based on data from the last patch test performed per individual. Data from the first patch test performed ignoring subsequent patch tests were used when examining the frequency of multiple contact allergies compared with age. It is stated in the Results section when data from the first patch test were used as point of origin.

Comparison of sensitivity rates and proportions was made with χ^2 tests and evaluation of trends over time with χ^2 for trend. The *p* value was adjusted according to the method of Bonferroni when comparing sex-specific sensitivity rates. In these instances, a *p* value below 0.002 was regarded as significant. For the remaining calculations in manuscript I and II a *p* value below 0.05 was regarded as significant. Comparison of sensitivity rates between tests on the same population was done with the McNemar non-parametric test based on binomial distribution. Comparison of age medians in manuscript I and age means in manuscript II was done with the Mann-Whitney test and independent-samples T-test, respectively. Levene´s test was used to examine equality of variances and Kolmogorov-Smirnov test for normal distribution.

The influence of sex and age on polysensitization was evaluated by nominal logistic regression models. Polysensitization was used as dependent outcome variable, and age, sex, and interac-

ALLERGENS	NT % (n)	NT:S % (n)	Missing data % (n)
Potassium dichromate	0.1% (20)	0.3% (41)	0
Neomycin sulphate	0.1% (22)	0.2% (27)	0
Thiuram mix	0.1% (19)	0.2% (40)	0
Phenylenediamine, para-	0.2% (35)	0.1% (23)	0
Cobalt chloride	0.2% (25)	0.3% (56)	0
Benzocaine	0.0% (3)	0.0% (4)	0
Formaldehyde	0.1% (19)	0.2% (29)	0
Colophony	0.1% (19)	0.4% (59)	0
Clioquinol	0.0% (2)	0.1% (11)	0
Isopropyl-N-phenyl PPD, N- (IPPD)	0.1% (15)	0.1% (11)	0.1% (20)
Wool Alcohols	0.0% (4)	0.1% (14)	0
Mercapto mix	0.1% (10)	0.1% (24)	0
Epoxy resin	0.1% (11)	0.1% (16)	0
Parabens mix	0.0% (3)	0.0% (3)	0
Tert.-but.phenolformaldehyde (BPF) resin, para-	0.0% (5)	0.1% (13)	0
Fragrance mix I with Sorbitan Sesquioleate (5%)	0.2% (31)	0.6% (90)	0
Myroxolon pereirae	0.1% (12)	0.3% (48)	0
Sesquiterpene lactone cocktail	2.2% (350)	0.1% (21)	10.0% (1604)
Quaternium-15	0.0% (6)	0.1% (9)	0
Nickel sulphate	1.0% (169)	1.3% (217)	0
(Cl)Me-isothiazolinone	0.8% (131)	0.1% (21)	0
Mercaptobenzothiazole (MBT)	1.0% (157)	0.1% (20)	0
Primin	0.1% (14)	0.1% (16)	0

TABLE 2: *Registrations of "Not Tested" (NT), "Not Tested: Sensitized" (NT:S), and missing data for each allergen in the European Baseline Series.*

tion between age and sex as independent variables. The nominal regression model was run with maximum likelihood estimation. The relative contribution of each allergen to polysensitization was evaluated by binary logistic regression analyses. Polysensitization was used as dependent outcome variable, and the particular allergen of interest as independent variable as well as sex, age-grouping and interaction between sex and age-grouping as covariables. One logistic analysis was made for each allergen. Hosmer-Lemeshow goodness-of-fit test was used to test if the binary logistic model fitted the data adequately.

Polysensitization in the logistic analyses in manuscript II corresponds to ≥ 2 additional positive reactions to baseline series allergens i.e. excluding the positive reaction to the allergen investigated. Mercaptobenzothiazole (MBT) and quaternium-15 were excluded from all counts of additional allergies to reduce the risk of duplicate counts of the same allergy (mercapto mix and MBT, and formaldehyde and quaternium-15).

3.2. STUDY PART II, MANUSCRIPT III

3.2.1. Study design and study subjects

The study was a dose-response study based on data from 3 previous investigations [59-61] that were re-analysed with the specific aim in mind; 53 test subjects were included based on a minimum patch test reaction of 1+ to nickel sulphate, methyldibromo glutaronitrile (MDBGN) or *p*-phenylenediamine (PPD). Positive reactions to other allergens in the European Baseline Series and a supplementary standard series were counted as additional allergies. All participants had been tested with both series as part of ordinary diagnostics at the same department. The individual combinations of allergens were evaluated and only allergens which were not chemically / structurally related were regarded as true allergies. Patients were then divided into two groups based on the number of registered contact allergies: patients with 1-2 contact allergies and patients with ≥ 3 contact allergies.

None of the test subjects had been treated with systemic immunosuppressive medications during the week before patch testing and no test subjects had been treated with topical steroids during the 2 weeks before patch testing. None of the test subjects had active eczema at the time of patch testing. Four persons tested with PPD had been exposed to UV light on their backs during the 3 weeks before patch testing: three from sun light, one from a sunbed. The remaining test subjects had not been exposed to UV light during the 3 weeks before the patch test. The PPD, nickel sulphate and MDBGN dose-response studies were conducted January-June, February-April, and March-August, respectively.

3.2.2. Patch test procedure

The patch tests were performed on the upper back using Finn Chambers® and Scanpor Tape® for all three allergens. Dilution series and controls used are illustrated in TABLE 3. A 48-hour occlusion was used except for one patient tested with MDBGN where the patch tests were removed after 24 hours due to intense itching. Readings were done by the principal investigators accompanied by specialized nurses on D2, D3/4 and D7. The reading on D3 was used for the statistical calculations in the present study. The different concentrations were applied on the

TABLE 3: *Concentrations and controls used in the dilution series for nickel sulphate, methyldibromo glutaronitrile (MDBGN) and p-phenylenediamine (PPD), respectively.*

	NICKEL SULPHATE	MDBGN	PPD
Dilution series:	3%	0.5%*	1.0%
	2.2%	0.34%*	0.5%
	0.75%	0.17%	0.1%
	0.375%	0.085%	0.05%
	0.22%	0.052%	0.01%
	0.075%	0.034%	0.005%
	0.05%	0.017%	0.001%
	0.022%	0.01228%	0.0001%
	0.01172%	0.0052%	
	0.0075%	0.0034%	
	0.005%	0.0017%	
	0.00293%	0.001228%	
	0.00146%	0.000614%	
	0.000732%	0.000307%	
	0.0005%	0.0001228%	
	0.000183%	0.0000614%	
	0.0000915%	0.0000307%	
	0.0000457%	0.00001535%	
	0.0000228%	0.0000077%	
Control:	10% ethanol / 90% water	20% ethanol / 80% water	white petrolatum

* Only 5 patients were tested with the 0.5% concentration of MDBGN, and 17 with the 0.34% concentration of MDBGN.

back in a randomised manner and readings were done blinded for nickel sulphate and MDBGN. Order of concentrations on the back and readings were not blinded in the PPD study.

The reading scale for nickel sulphate and MDBGN was an extension of a previously developed reading scale [62] and consisted of 9 scale steps. A reading of 2 and above was considered a positive response. The reading scale of PPD consisted of 5 scale steps and was based on the International Contact Dermatitis Research Group Criteria [58]. A reading of 1 and above was considered a positive response. The reading scales are illustrated in TABLE 4. The threshold concentration was defined as the weakest concentration giving a positive response on D3 in a continuous line of patch test reactions starting from the highest concentration. Reactions less than a standard 1+ were considered positive in this setting as the test subjects were verified sensitized and such reactions were registered in succession with 1+/2+/3+ reactions.

3.2.3. Data analyses

A logistic dose-response model equivalent to the distribution of the threshold doses [63] was estimated from the observed threshold dose-response data by means of asymptotic maximum likelihood methods using statistical software developed in APL*PLUS®, STSC Inc, Rockville, Maryland, USA. The statistical analysis comprised likelihood ratio tests (χ^2) of goodness of fit, estimation of pairs of parallel logistic threshold dose-response curves, tests of parallelism and calculation of relative sensitivity with a 95% confidence interval (CI 95%). Parallel response versus $\log_{dose}$ relations are required for expressing the relative sensitivity or potency as a single number [63]. The relative sensitivity describes the horizontal displacement of the two dose-

response curves and corresponds to the ratio between doses that elicit positive response in the same fraction of the subjects, e.g. ED_{50}. Statistical tests were regarded as significant if $p \leq 0.05$.

Independent samples T-test compared age means. The observations were independent. Assumption of Normality was tested with Kolmogorov-Smirnov test and Levene´s Test was used to test for equality of variances. Comparison of sex distribution was done with Fisher's exact test.

3.3. STUDY PART III, MANUSCRIPT IV & V

3.3.1. Study design

This was a case-control study. A questionnaire was mailed to cases and controls together with an introductory letter and pre-paid return envelope. The response rate was increased with a second recruit procedure.

3.3.2. Study subjects and matching procedures

The study population was nested in the hospital patch test population used for study part I. The flow of patients is illustrated in FIGURE 1. Of 759 polysensitized patients identified in the database population, 562 were still alive and could be located, had not emigrated and were ≥ 18 years at the time of survey. They were matched individually in a 1:2 order with patients with 1-2 contact allergies also identified in the database population. The total study population reached 1686 subjects: 562 polysensitized and 1124 with 1-2 contact allergies. Matching parameters were sex, age ± 2 years and time of patch test ± 24 months. Matching was performed with the computer program SQL Query Analyzer® version 8.00.194 (Microsoft Corporation, Redmond, Washington, USA). All suitable controls were listed for each case. Two controls with the closest

NICKEL SULPHATE †	MDBGN †	PPD´¶
0 = No reaction	0 = No reaction	0 = No reaction
1 = Few papules with no erythema, no infiltration	1 = Few papules with no erythema, no infiltration	1 = Follicular reaction
2 = Faint erythema with no infiltration or papules	2 = Faint erythema with no infiltration or papules	2 = Faint erythema without infiltration or papules (+?)
3 = Faint erythema with few papules and no homogenous infiltration	3 = Faint erythema with few papules and no homogenous infiltration	3 = Homogenous redness and infiltration covering the whole test area (+)
4 = Erythema, homogenous infiltration	4 = Erythema, homogenous infiltration	4 = Homogenous redness, infiltration, papules and vesicles (++)
5 = Erythema, infiltration and a few papules	5 = Erythema, infiltration and a few papules	5 = Homogenous redness, infiltration and coalescing vesicles (+++)
6 = Erythema, infiltration and papules	6 = Erythema, infiltration and papules	
7 = Erythema, infiltration, papules and a few vesicles	7 = Erythema, infiltration, papules and a few vesicles	
8 = Intense erythema, infiltration, vesicles	8 = Intense erythema, infiltration, vesicles	
9 = Bulla	9 = Bulla	

† A score of 2 or above was considered a positive response for nickel sulphate and MDBGN.

¶ A score of 1or above was considered a positive response for PPD.

TABLE 4: *Reading scales used in the three dose-response studies for nickel sulphate, methyldibromo glutaronitrile (MDBGN) and p-phenylenediamine (PPD), respectively.*

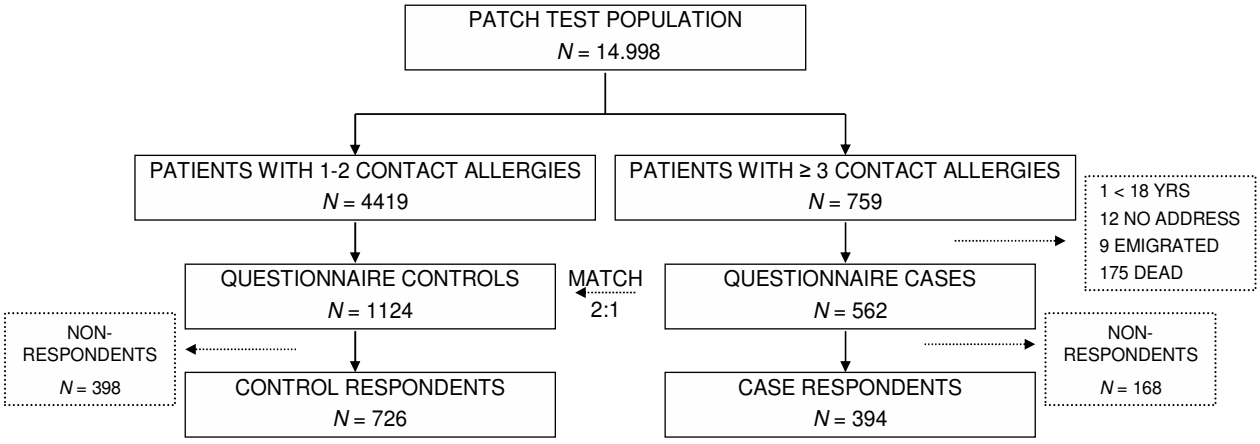

FIGURE 1: *Flow of patients through study parts*

match on age but still within the chosen boundaries for patch test year and still alive and living in Denmark were chosen for each case. Once a control was chosen, the control was withdrawn from the matching process and could not be matched with other cases. For one control, age diverged by 3 years; for 6 controls, time of patch test diverged by 26-48 months.

3.3.3. The questionnaire

The questionnaire consisted of 70 items; mainly fixed-response questions but also some open-ended questions where needed. The items covered aspects of self-reported dermatitis, work, education, contact allergies and patch testing, general health and other skin diseases, multiple chemical sensitivities and dermatitis in straight-line relatives. Most questions used in this study part were modified from questions in previous questionnaires developed to survey hand dermatitis and collected in the Nordic Occupational Skin Questionnaire 2002 (NOSQ-2002) [64]. The question on self-reported diagnosis of dermatitis was modified from NOSQ question D1. Questions on debut and cessation of dermatitis were modified from NOSQ D5 and D6. Questions on other skin diseases were modified from NOSQ-2002 U1. The question used to determine outbreak frequency and site of dermatitis was constructed for the present survey. The response categories for the outbreak frequency-question were identical with the response categories for a similar question in other questionnaires [65;66]. Questions on school and vocational education were identical with questions in other questionnaires used in the Danish population [67]. The UK Working Party´s Diagnostic Criteria, question-only version, identified patients with atopic eczema [68].

3.3.4. Definitions

A *diagnosis of dermatitis* was defined as “yes” to the question: “Have you ever had dermatitis?”. A *diagnosis of other skin diseases* was defined as “yes” to the question: “Have you ever had one of the following skin diseases: psoriasis, itch without visible skin lesions, urticaria, leg ulcers, or other skin disease, please specify”. The *duration of disease* was measured in years by

subtracting the debut year from the year where last dermatitis episode occurred. The duration of disease measures the total duration between first and last dermatitis episode regardless of intermittent dermatitis-free episodes. *Age at onset* was measured in years by subtracting the birth year from the year of debut of dermatitis. *Outbreak frequency* was determined by asking whether the dermatitis occurred intermittently and if so, how much time between first and last dermatitis episode had been free of dermatitis. Four options were given: if the person had been free of dermatitis for more than ½ of the time, about ½ of the time, less than ½ of the time, or none of the time (= *persistent dermatitis*). *Site of dermatitis* was determined by asking where the dermatitis was located at time of debut. The body surface was divided into 19 different skin areas: scalp, periorbital region, periauricular region, perioral region, remaining part of face, neck, shoulders, armpits, cubital folds, arms excluding hands, hands and/or wrists, chest, back, stomach, buttocks, popliteal folds, legs excluding feet, feet and/or ankles, anogenital region. The number of skin areas that could be marked as affected with dermatitis at time of debut was not restricted.

Educational level was based on years of education. The Danish Educational Nomenclature, developed by the organisation Statistics Denmark and Danish Ministry of Education, was used to classify each specific education into educational levels [69].

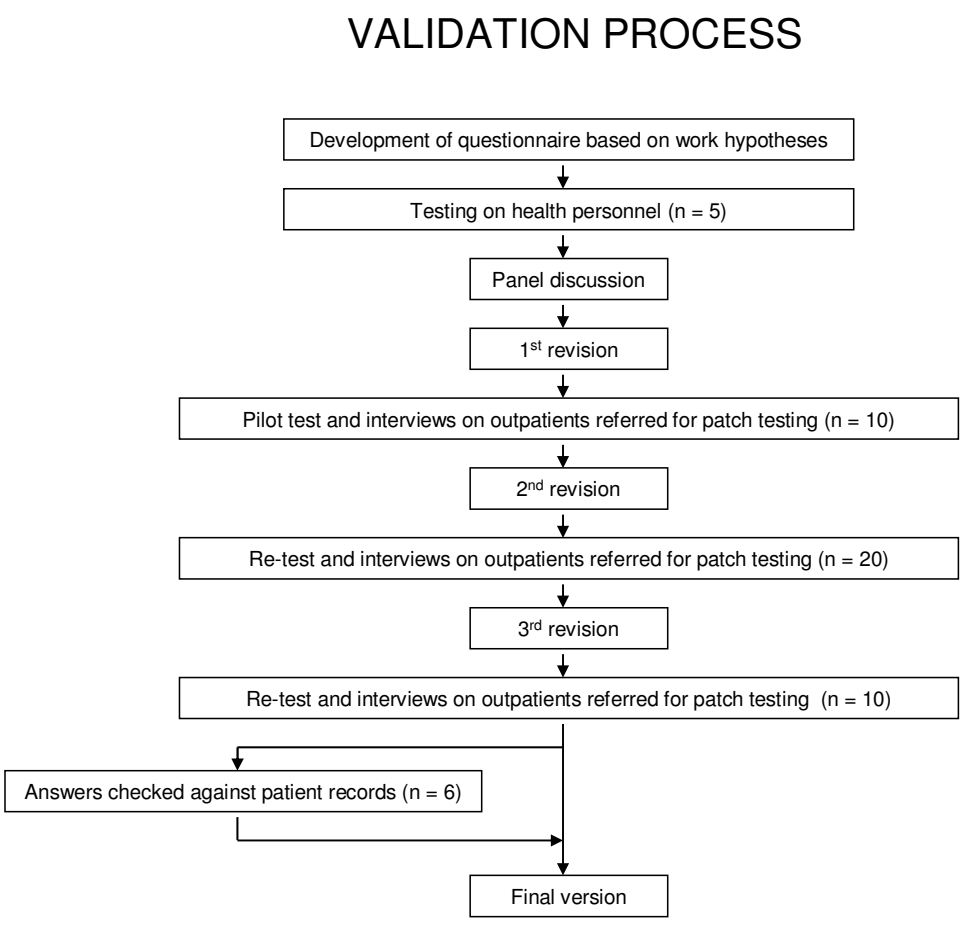

FIGURE 2: *Validation of questionnaire*

3.3.5. Validation of questionnaire

The validation process is illustrated in FIGURE 2. The questionnaire was developed by the head investigator (BC) and supervisors. Initially, five health personnel, one with atopic eczema, evaluated the questionnaire and participated in a panel discussion. They commented on any major problems related to structure, wording and response categories. The questionnaire was revised and re-evaluated in a pilot test conducted in 10 consecutive outpatients undergoing patch testing. They answered the questionnaire and commented on all aspects of the questionnaire e.g. layout, structure, questions, wording and response categories. The completed questionnaires were looked through by the head investigator to identify any problems not revealed spontaneously by the test subjects. Floor/ceiling-effects were assessed to ensure the questions could measure that intended. Comparison of responses to questions expected to correlate was performed where possible. A second revision and re-evaluation of the questionnaire was done on additional 20 consecutive outpatients referred to patch testing. Finally, a third revision and re-evaluation was done on 10 consecutive outpatients. In 6 of the 10 final test patients, answers eligible to be counterchecked in patient records were performed. In the final test, the questions and response categories functioned well and were considered easy to understand and relevant for the test subjects.

3.3.6. Data entering and validation

The data were entered manually into a database exclusively by the head investigator (BC) using SPSS Data Entry Builder® (SPSS Inc., Chicago, Illinois, USA). Typing error was checked by retyping 60 questionnaires equivalent to 5% of all typed questionnaires. A built-in control function in the computer program was used to check for discrepancies between the double-typed questionnaires. The percentage of error was 1.1 ‰. Each variable was checked for outlying parameters and missing data by frequency tables, and internal consistency was checked by extensive cross tabulations. In the case of errors or inconsistencies, values were checked against the original questionnaires. The frequency of missing data varied with the questions from 0.5% for self-reported dermatitis to 6.2% for duration of disease (TABLE 5).

3.3.7. Data analyses

The statistical analyses were performed in SPSS® software version 15.0 (SPSS Inc., Chicago,

Questions	Missing data
Atopic eczema	1.4%
Self-reported dermatitis	0.5%
Site of dermatitis	1.4%
Number of skin areas affected	1.4%
Duration of disease	6.2%
Age at onset	3.8%
Course of disease	4.3%
Educational level	5.4%
Other skin diseases *	3.2%

* Missing data for question regarding leg ulcers and other skin diseases

TABLE 5: *Missing data for questionnaire study. Number of participants = 1120.*

Illinois, USA). Patients with incidental missing data were excluded from analyses concerning that particular variable. A minor part of the total population reported never having had dermatitis and were excluded from analyses concerning clinical characteristics.

Comparisons of frequencies and sensitivity rates were made by χ^2 tests and evaluation of trends with χ^2 for trend. Comparison of mean age was done with independent samples T test. Assumptions of Normality were checked by Kolmogorov-Smirnov tests and assumption of equal variances by Levene's test. Comparison of median age at debut, median duration of disease, median number of skin areas affected was done with the Mann Whitney test. Normal distribution of continuous data was checked with Kolmogorov-Smirnov tests.

The association between polysensitization and atopic eczema (AE), educational level, duration of disease, outbreak frequency and leg ulcers was evaluated by one binary logistic regression model. Another binary logistic regression model evaluated the association between polysensitization and specific skin sites affected with dermatitis at debut. Three analyses based on a population consisting of all respondents, a population of patients with AE and a population of patients without AE, respectively, were performed for each model. Polysensitization was used as dependent outcome variable for each analysis. Sex, age, AE and time of patch test were included as co-factors for the analyses based on the total population, and age, sex, and patch test year were included as co-factors for analyses concerning subpopulations according to AE status. Hosmer-Lemeshow goodness-of-fit test was used to test if the model fitted the data adequately.

Correlations between the different skin sites were examined by multiple 2^x2 tables for two different strata: individuals with and without AE and hypothesis tested with χ^2-tests. These calculations and comparison of sensitivity rates for European Baseline Series allergens in the drop-out analysis were adjusted according to the method of Bonferroni. A *p* value below $2.9 \cdot 10^{-4}$ and 0.002, respectively, was regarded as significant in these instances. For the remaining calculations a *p* value below 0.05 was regarded as significant.

4. RESULTS

4.1. STUDY PART I, MANUSCRIPT I & II

4.1.1. Descriptive data

Characteristics of the entire study population regarding sex and age distribution and sensitivity rates are displayed in TABLE 6. The overall median age at first patch test was 47.4 years (interquartile range (IQR) 28.9). Demographics for patients with 1-2 contact allergies and patients with ≥ 3 contact allergies are displayed in TABLE 7.

4.1.2. Sensitivity rates and trends

The overall sensitivity rate reached 34.5% (5178), range 1-12 contact allergies; 5.1% (759) had multiple contact allergies and 4419 (29.5%) had 1-2 contact allergies based on the last patch test performed. The proportion of patients with 1-2 contact allergies, patients with ≥ 3 contact allergies and negative cases remained stable over 20 years (FIGURE 3) ($p = 0.647$), despite a steady increase in the total number of patients patch tested each year (FIGURE 4). From 1985-2005, the age median increased by approximately five years. The fraction of women tested each year varied overall, showing only a marginal increase from 1985 to 2005 (FIGURE 4).

4.1.3. Patients with multiple contact allergies

At the time of the first patch test 683 individuals had multiple contact allergies, correlating to 90% of all patients diagnosed with multiple allergies. The median age at time of diagnosis of multiple contact allergies was 53.0 years (IQR 26.5). The frequency of patients with multiple contact allergies increased with age (FIGURE 5). The frequency of 1-2 allergies did not show the same increasing trend with age (results not shown). The occurrence of multiple contact allergies was significantly associated with sex and age and sex*age in combination (all factors, $p < 0.0001$).

11.2% (77/683) of patients diagnosed with multiple contact allergies and 7.6% of patients diagnosed with 1-2 contact allergies at time of first test ended up being tested multiple times ($p < 0.001$). The proportion of multiple-allergic patients among individuals tested several times increased from 7.9% (77/977) at the first patch test to 13.8% (135/977) at the second patch test to 24.1% (28/116) at the third patch test. The increase between first and second test was significant ($p < 0.001$). The proportion of patients with 1-2 contact allergies also increased with renewed testing (first patch test 34.2% (334/977), second patch test 36.7% (359/977), third patch test 40.5% (47/116)).

4.1.4. Allergen-specific prevalence rates

Nickel sulphate was the most frequent allergen in patients with 1-2 contact allergies followed by fragrance mix and Myroxylon pereirae. Fragrance mix was the most frequent allergen in patients with multiple contact allergies followed by nickel sulphate and Myroxylon pereirae. Almost every second patient with multiple allergies was allergic to fragrance mix and 43.8% were allergic to nickel sulphate. Allergen-specific sensitivity rates for the total population, for single/double-sensitized and for polysensitized individuals, respectively, are listed in TABLE 8.

VARIABLE	CATEGORY	*N*	PER CENT
Sex	Female	9545	63.6%
	Male	5453	36.4%
Age at first patch test	< 16 years	255	1.7%
	16-30 years	3041	20.3%
	31-50 years	5212	34.8%
	51-70 years	4437	29.6%
	> 70 years	2053	13.7%
Multiple tests	2-5	977	6.5%
Prevalence	0 contact allergies	9820	65.5%
	1-2 contact allergies	4419	29.5%
	≥ 3 contact allergies	759	5.1%

TABLE 6: *Characteristics of the total study population (n = 14,998) in the database study*

	1-2 contact allergies (*n* = 4419)	**≥ 3 contact allergies (*n* = 759)**	***p* value**
Female %	73.0%	77.3%	$p = 0.013$
Median age (IQR)	47.9 yrs (26.9)	53.3 yrs (26.0)	$p < 0.001$

IQR = interquartile range

TABLE 7: *Demographic comparison of patients with 1-2 contact allergies and ≥ 3 contact allergies based on the last patch test performed per individual.*

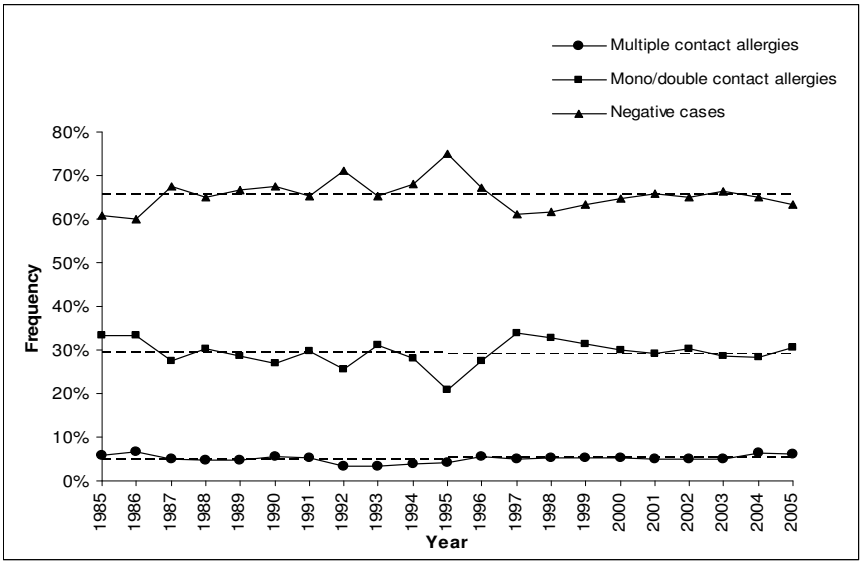

FIGURE 3: *Prevalence of negative, single/double-sensitized and polysensitized individuals over 20 years*

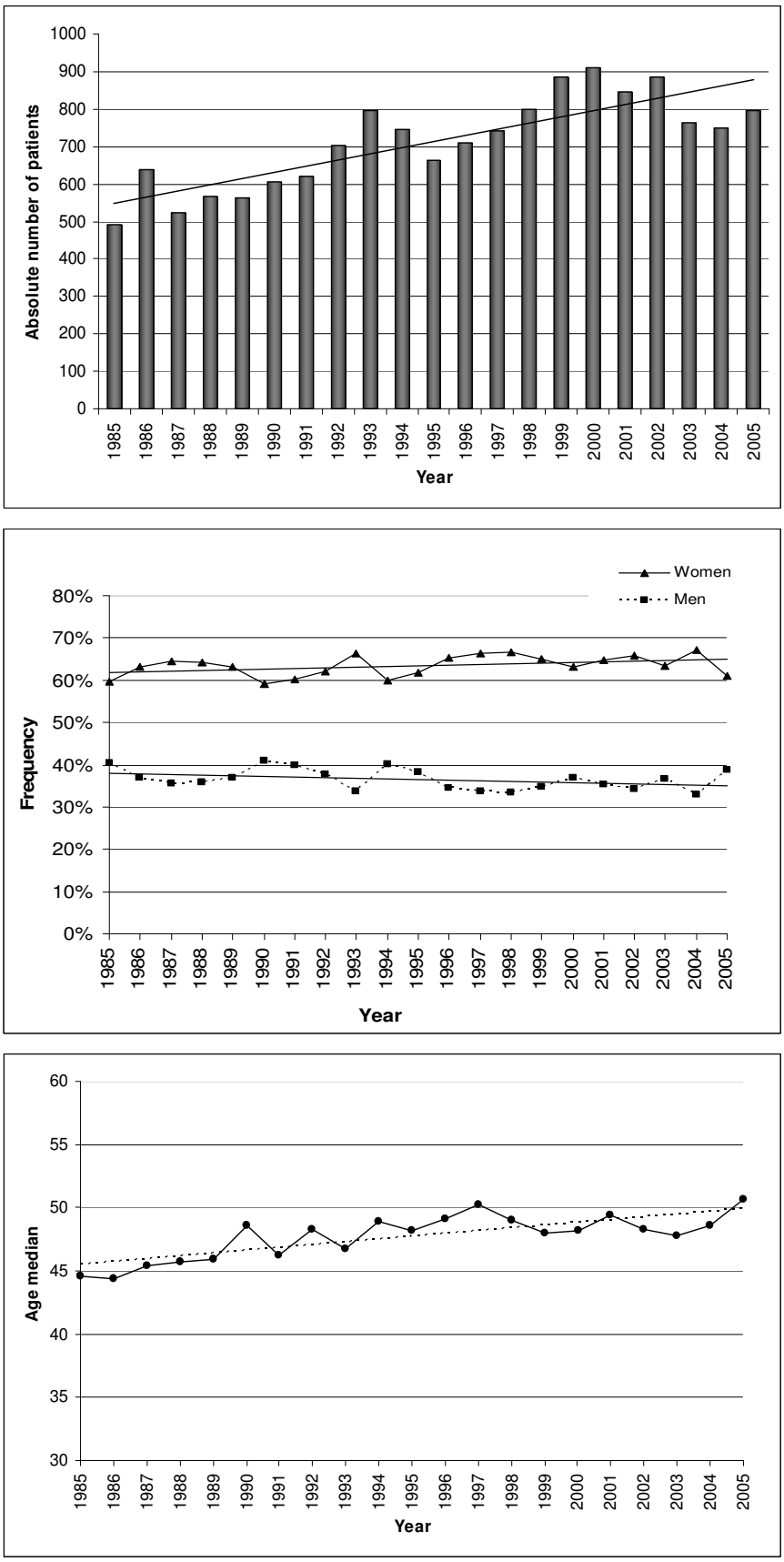

FIGURE 4: *A: Number of patients patch tested each year during 1985-2005. B: Sex distribution in the patch test population during 20 years. C: Changes in age median in the patch test population over 20 years.*

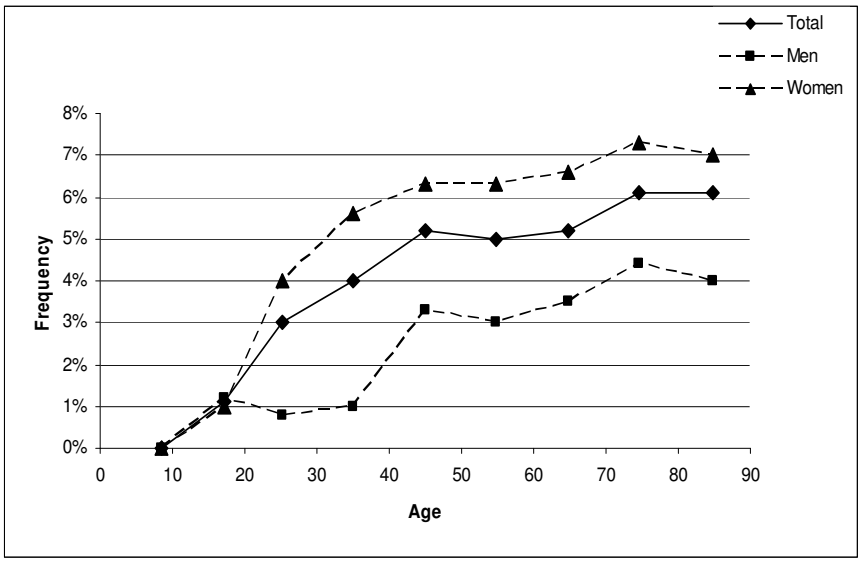

FIGURE 5: *Frequency of multiple contact allergies in relation to age and sex at time of first patch test in a Danish patch test population*

ALLERGENS	**TOTAL POPULATION (*n* = 14.998)**		**1-2 CONTACT ALLERGIES (*n* = 4419)**		**≥ 3 CONTACT ALLERGIES (*n* = 759)**	
	Positive reactions (*n*)	Sensitivity rate (%)	Positive reactions (*n*)	Absolute frequency (%)	Positive reactions (*n*)	Absolute frequency (%)
Fragrance mix I	1157 (14971)	7.7 *	788 (4410)	17.9	369 (755)	**48.9**
Nickel sulphate	1785 (14845)	12.0 *	1459 (4376)	33.3	326 (744)	**43.8**
Myroxylon pereirae	658 (14988)	4.4	391 (4416)	8.9	267 (758)	**35.2**
Colophony	583 (14985)	3.9 *	365 (4414)	8.3	218 (758)	**28.8**
Cobalt chloride	544 (14976)	3.6 *	346 (4416)	7.8	198 (756)	**26.2**
Formaldehyde	428 (14980)	2.9 *	265 (4415)	6.0	163 (756)	**21.6**
Potassium dichromate	363 (14979)	2.4	206 (4415)	4.7	157 (756)	**20.8**
Thiuram mix	403 (14982)	2.7	263 (4415)	6.0	140 (758)	**18.5**
MCI/MI	264 (14878)	1.8 *	157 (4387)	3.6	107 (748)	**14.3**
Neomycin sulphate	420 (14978)	2.8	313 (4414)	7.1	107 (756)	**14.2**
p-phenylenediamine	319 (14966)	2.1	224 (4409)	5.1	95 (756)	**12.6**
SL mix	159 (13198)	1.2 *	82 (3869)	2.1	77 (656)	**11.7**
Quaternium 15	131 (14993)	0.9 *	58 (4418)	1.3	73 (757)	**9.6**
Wool alcohols	136 (14994)	0.9	70 (4418)	1.6	66 (759)	**8.7**
Mercapto mix	100 (14989)	0.7	38 (4415)	0.9	62 (758)	**8.2**
PTBFR	182 (14994)	1.2 *	124 (4417)	2.8	58 (759)	**7.6**
Mercaptobenzothiazole	78 (14852)	0.5	24 (4378)	0.5	54 (744)	**7.3**
Primin	168 (14986)	1.1 *	115 (4417)	2.6	53 (755)	**7.0**
Clioquinol	110 (14996)	0.7	63 (4418)	1.4	47 (759)	**6.2**
Epoxy resin	166 (14987)	1.1	123 (4413)	2.8	43 (758)	**5.7**
Parabens mix	83 (14996)	0.6	42 (4417)	1.0	41 (759)	**5.4**
IPPD	79 (14964)	0.5	40 (4410)	0.9	39 (756)	**5.2**
Benzocaine	82 (14995)	0.5	53 (4419)	1.2	29 (758)	**3.8**
Total	8398		5609		2789	

*marks the allergies that are overrepresented among women.

IPPD =*N*- isopropyl-*N*-phenyl-*p*-phenylenediamine, MCI/MI = methylchloroisothiazolinone/methylisothiazolinone, PTBFR = *p*-tertiary butylphenol formaldehyde resin, SL mix = sesquiterpene lactone mix

TABLE 8: *Positive reactions and absolute sensitivity rates for each allergen in the European Baseline Series for the total population, a single/double-sensitized and polysensitized group, respectively.*

4.1.5. Association between polysensitization and allergens in the EBS

SL mix, parabens mix, IPPD, and wool alcohols, constituted the greatest risk of being part of a complex of multiple contact allergies (odds ratio (OR) 1.7, OR 1.7, OR 1.6 and OR 1.5; respectively). Myroxylon pereirae, potassium dichromate and cobalt chloride were also positively associated with polysensitization (OR 1.4, OR 1.4, OR 1.3, respectively). PPD, neomycin sulphate, epoxy resin, primin and nickel sulphate showed a negative association with polysensitization (OR 0.8, OR 0.6, OR 0.6, OR 0.6, OR 0.5, respectively). The results from the regression analyses are presented in TABLE 9.

The absolute frequency of ≤ 1 and ≥ 2 additional contact allergies given a specific contact allergy to one of 21 standard allergens is shown in TABLE 10. For the allergens with the strongest associations, every second individual had ≥ 2 additional allergies.

4.2. STUDY PART II, MANUSCRIPT III

4.2.1. Descriptive data

Descriptive data for the group of patients with multiple contact allergies and 1-2 contact allergies, respectively, are presented in TABLE 11.

ALLERGENS	N	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
Myroxylon pereirae	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
***p*-phenylenediamine**	5147	**0.8**	0.6 – 0.97
PTBFR	5158	0.8	0.6 – 1.2
Neomycin sulphate	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 sulphate	5102	**0.5**	0.4 – 0.6

† adjusted for sex, age and interaction between sex and age

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

TABLE 9: *Risk of having ≥ 2 additional contact allergies compared with ≤ 1 additional contact allergy provided a given allergy to 1 of 21 allergens in the European Baseline Series. Statistically significant associations are printed in* ***bold****.*

ALLERGENS (N)	≤ 1 ADDITIONAL CONTACT ALLERGY	≥ 2 ADDITIONAL CONTACT ALLERGIES
Parabens mix (83)	42 (50.6%)	41 (49.4%)
Sesquiterpene lactone 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%)
Myroxylon pereirae (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%)
p-phenylenediamine (319)	228 (71.5%)	91 (28.5%)
Epoxy resin (166)	123 (74.1%)	43 (25.9%)
Neomycin sulphate (420)	316 (75.2%)	104 (24.8%)
Nickel sulphate (1785)	1472 (82.5%)	313 (17.5%)

IPPD = *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine MCI/MI = methylchloroisothiazolinone/methylisothiazolinone
PTBFR = *p*-tertiary butylphenol formaldehyde resin

TABLE 10: *Frequency of additional allergies given a specific contact allergy to 1 of 21 allergens in the European Baseline Series.*

	1-2 contact allergies (*n* = 38)	≥ 3 contact allergies (*n* = 13)	*p* value
Mean age in years (± SD)	43.6 (± 13.4)	45.8 (± 10.4)	$p = 0.603$
Female % (*n*)	89.5% (34)	84.6% (11)	$p = 0.638$

SD = standard deviation

TABLE 11: *Descriptive data for the group of patients with single/double-sensitizations and polysensitizations, respectively, in the dose-response study.*

4.2.2. Nickel sulphate

Twenty nickel-allergic persons were patch tested with a nickel sulphate dilution series; 1 person did not react to any of the nickel sulphate solutions and was excluded from further analysis. Of the remaining 19 test subjects, 10 had no additional contact allergies, 6 had 1 additional contact allergy, and 3 had 2 additional contact allergies. The lowest threshold concentration observed in the polysensitized group was 0.022% and 0.01172% in the single/double-sensitized group. None of the nickel-allergic patients reacted to the ethanol/water control. The parallel dose-response curves for nickel sulphate are shown in FIGURE 6.

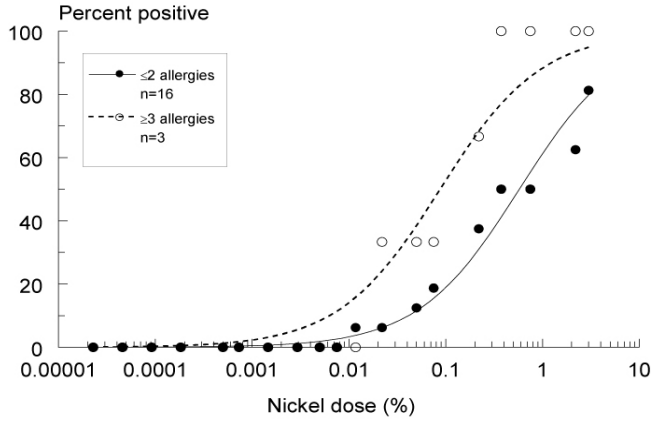

FIGURE 6: *Nickel sulphate dose-response curve for single/double-sensitized and polysensitized patients, respectively, with the accumulated frequency of patients plotted against the log concentration tested.*

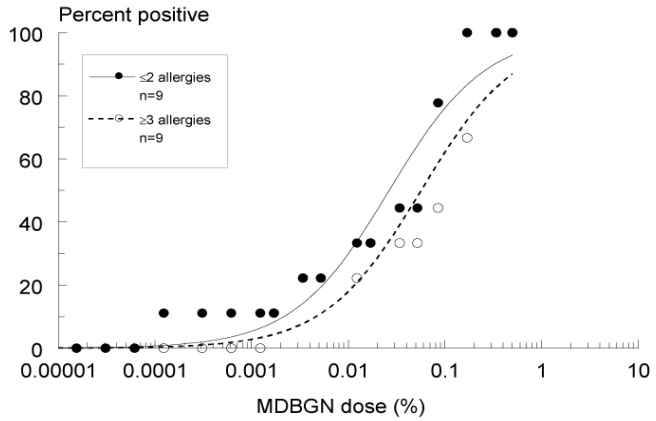

FIGURE 7: *Methyldibromo glutaronitrile (MDBGN) dose-response curve for single/double-sensitized and polysensitized patients, respectively, with the accumulated frequency of patients plotted against the log concentration tested.*

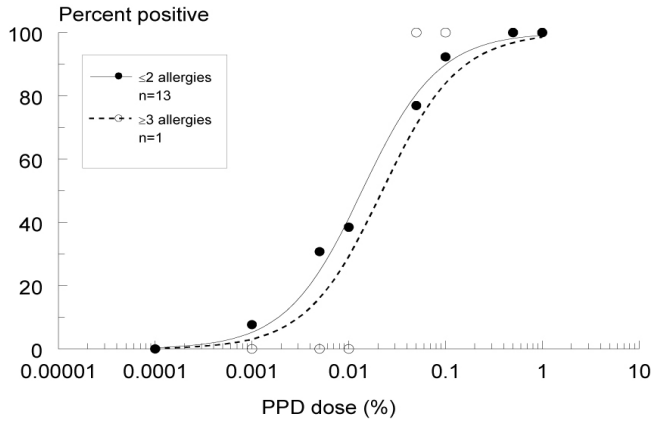

FIGURE 8: *p-phenylenediamine (PPD) dose-response curve for single/double-sensitized and polysensitized patients, respectively, with the accumulated frequency of patients plotted against the log concentration tested.*

4.2.3. Methyldibromo glutaronitrile

Eighteen MDBGN-allergic persons were patch tested with a MDBGN dilution series. Of these, 4 persons had no additional contact allergies, 5 had 1 additional contact allergy, and 9 had ≥ 2 additional contact allergies. The lowest threshold concentration observed in the polysensitized group was 0.0017% and 0.0001228% in the single/double-sensitized group. One of the MDBGN-allergic patients reacted with a few papules but no erythema and no infiltration to the ethanol/water control. This person also reacted with a few papules without erythema and infiltration at other test chamber sites which were not in succession with 1+/2+/3+ MDBGN reactions. The threshold concentration for this patient was 0.17%. The parallel dose-response curves for MDBGN are shown in FIGURE 7.

4.2.4. *p*-phenylenediamine

Fifteen PPD-allergic persons were patch tested with a PPD dilution series; 1 person did not react to any of the PPD solutions and was excluded from further analysis. Of the remaining 14 test subjects, 10 had no additional contact allergies, 3 had 1 additional contact allergy, and 1 had 3 additional contact allergies. The lowest threshold concentration observed in the polysensitized group was 0.05% and 0.001% in the single/double-sensitized group. None of the PPD-allergic patients reacted to the petrolatum control. The parallel dose-response curves for PPD are shown in FIGURE 8.

4.2.5. Relative sensitivity

The relative sensitivity was defined as the ratio between the dose eliciting a reaction in 50% (ED_{50}) of the single/double-sensitized versus the polysensitized group. The relative sensitivity was estimated for each allergen separately and for the allergens combined and is shown with 95% confidence intervals (CI 95%) in TABLE 12. The relative sensitivities for the three allergens were identical ($p = 0.46$), which made it possible to summarize the relative sensitivities for each allergen into one combined relative sensitivity. The combined relative sensitivity was 68% (CI 95%: 19-251). There was no significant difference in sensitivity between the single/double- and polysensitized group as the confidence interval included 100%.

	NUMBER OF TEST SUBJECTS			
ALLERGEN	1-2 contact allergies	≥ 3 contact allergies	**Relative sensitivity (%)**	**95% CI**
Nickel	16	3	658	57 - 31377
MDBGN	9	9	47	4 - 293
PPD	13	1	62	2 - 1334
Total	38	13	68	19 - 251
Test for same sensitivity: $\chi^2(2) = 1.50$, P = 0.46				

MDBGN = methyldibromo glutaronitrile PPD = p-phenylenediamine CI = confidence interval

TABLE 12: *Relative sensitivity (ED_{50} (group with 1-2 contact allergies) / ED_{50} (group with ≥ 3 contact allergies)) for each allergen separately and for the allergens summed.*

TABLE 13: *Drop-out analysis for the questionnaire study*

	RESPONDENTS n = 1120 (66.4%)	NON-RESPONDENTS n = 566 (33.6%)	p value
Mean age (years ± SD)	47.6 ± 14.5	49.2 ± 15.9	$p = 0.052$
Female sex (%)	915 (81.7%)	453 (80.0%)	$p = 0.41$
Polysensitization (%)	394 (35.2%)	168 (29.7%)	$p = 0.024$

SD = standard deviation

4.3. STUDY PART III, MANUSCRIPT IV & V

4.3.1. Response rate

The questionnaire was returned by 1120 persons, corresponding to a response rate of 66.4%. Of these 1120 respondents, 394 had multiple contact allergies and 726 had 1-2 contact allergies. The questionnaire was completed by 70.1% (394/562) of the polysensitized individuals and 64.6% (726/1124) of the single/double-sensitized individuals ($p < 0.05$).

4.3.2. Drop-out analysis

Drop-out analysis is presented in TABLE 13. More individuals patch tested in the recent years than in the first years responded to the questionnaire, FIGURE 9 ($p < 0.001$). More respondents compared with non-respondents had contact allergy to potassium dichromate, fragrance mix I and quaternium-15 (results not shown), which became non-significant after adjustment for multiple testing.

4.3.3. Descriptive data

Comparisons between polysensitized and single/double-sensitized respondents regarding mean age and sex distribution, occurrence of dermatitis, atopic eczema, other skin diseases, and edu-

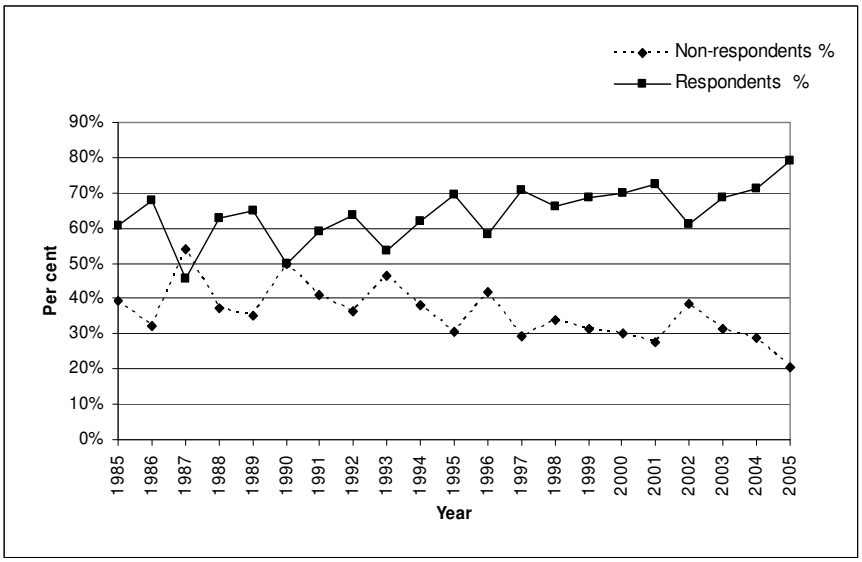

FIGURE 9: *Distribution of respondents and non-respondents according to patch test year*

cational level are illustrated in TABLE 14. The same percentage of polysensitized and single/double-sensitized respondents was patch tested each year between 1985 and 2005 (results not shown). The majority of respondents (983/1114) had had dermatitis at some point in time in their lives (88.2%); 397/1104 (36.0%) respondents had or had had atopic eczema (AE).

Variable	Category	1-2 contact allergies	≥ 3 contact allergies	*p* value
Sex	Female	81.1% (589)	82.7% (326)	$p = 0.51$
Age (mean ± SD)		47.5 ± 14.4	47.8 ± 14.8	$p = 0.767$
Educational level	Lowest level (≤ 10 years)	11.6% (84)	15.0% (59)	$p > 0.1$
	Low level (11-12 years)	38.2% (277)	35.3% (139)	$p > 0.2$
	Basic level (13-14 years)	10.5% (76)	13.7% (54)	$p > 0.1$
	Medium level (15-16 years)	21.3% (155)	21.6% (85)	$p > 0.2$
	High level (≥ 17 years)	9.1% (66)	7.4% (29)	$p > 0.2$
	EL unknown/ongoing	9.4% (68)	7.1% (28)	$p > 0.1$
Skin disease	Ever dermatitis	85.5% (618)	93.4% (365)	$p < 0.001$
	Atopic eczema	31.0% (221)	45.1% (176)	$p < 0.001$
	Leg ulcers	6.7% (47)	8.6% (33)	$p = 0.27$

SD = standard deviation

TABLE 14: *Descriptive data in the questionnaire study*

Status of AE	Variable	Category	1-2 contact allergies	≥ 3 contact allergies	*p* value
+ AE	Duration of disease (median, IQR)		29.0 years, IQR 25.0	33.0 years, IQR 23.25	$p = 0.11$
	Outbreak frequency	Dermatitis entire period	24.9% (53)	38.5% (67)	$p < 0.01$
		Dermatitis > ½ period	25.4% (54)	20.7% (36)	$p > 0.2$
		Dermatitis = ½ period	14.1% (30)	17.8% (31)	$p > 0.2$
		Dermatitis < ½ period	32.4% (69)	23.0% (40)	$p < 0.05$
	Age at onset (median, IQR)		18.0 years, IQR 28.0	18.0 years, IQR 26.0	$p = 0.473$
	Number of skin sites affected (median, IQR)		3.0, IQR 3.0	3.0, IQR 4.0	$p = 0.68$
No AE	Duration of disease (median, IQR)		15.5 years, IQR 22.75	22.0 years, IQR 20.75	$p = 5.7 \cdot 10^{-5}$
	Outbreak frequency	Dermatitis entire period	22.1% (88)	17.3% (33)	$p > 0.1$
		Dermatitis > ½ period	25.6% (102)	27.2% (52)	$p > 0.2$
		Dermatitis = ½ period	14.0% (56)	12.0% (23)	$p > 0.2$
		Dermatitis < ½ period	32.6% (130)	38.2% (73)	$p > 0.1$
	Age at onset (median, IQR)		31.0 years, IQR 30.0	29.5 years, IQR 27.0	$p = 0.019$
	Number of skin sites affected (median, IQR)		2.0, IQR 2.0	2.0, IQR 2.0	$p = 0.82$

AE = atopic eczema, IQR = interquartile range

TABLE 15: *Clinical characteristics*

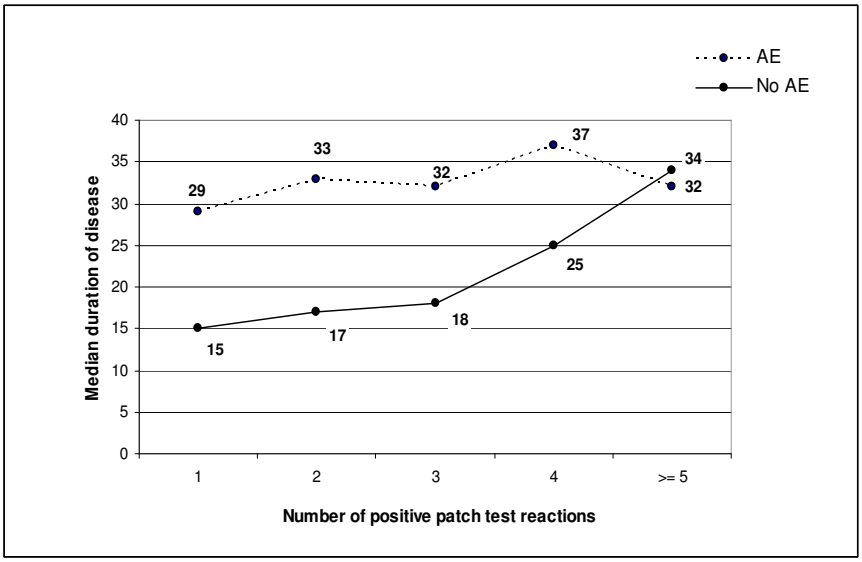

FIGURE 10: *Duration of disease according to number of positive patch test reactions for a group with and without atopic eczema (AE), respectively.*

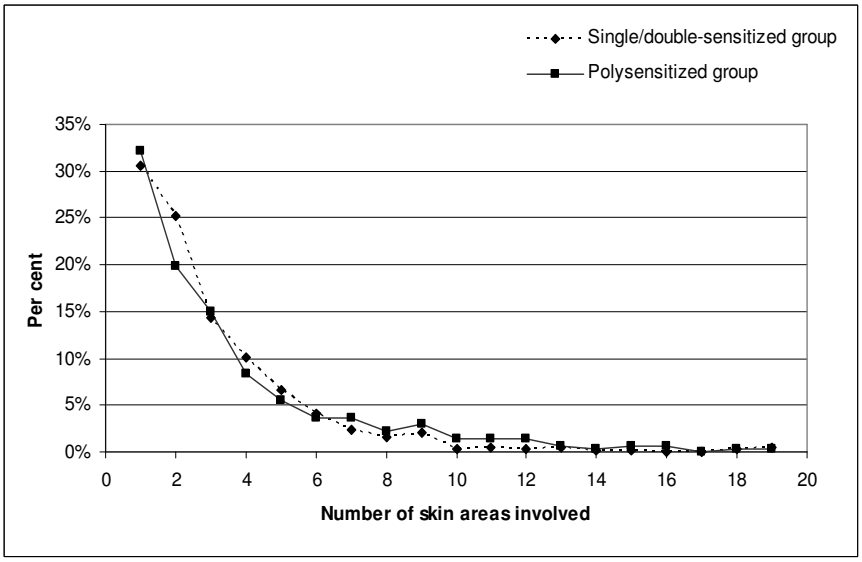

FIGURE 11: *Number of skin areas affected with dermatitis at time of debut compared with sensitization status*

4.3.4. Characteristics of clinical disease

Duration of disease, outbreak frequency, age at onset and number of skin areas affected at time of debut were estimated for the group of patients with multiple contact allergies and 1-2 contact allergies, respectively, divided according to status of AE. The results are shown in TABLE 15. For the group without AE the median duration of disease increased with the number of contact allergies (FIGURE 10). A similar relationship was not seen for the group with AE (FIGURE 10). FIGURE 11 illustrates the number of skin areas affected at debut compared with sensitization status.

SKIN SITE	- ATOPIC ECZEMA (N=580) Single/double-sensitized individuals % (N=393)	- ATOPIC ECZEMA (N=580) Polysensitized individuals % (N=187)	+ ATOPIC ECZEMA (N=385) Single/double-sensitized individuals % (N=212)	+ ATOPIC ECZEMA (N=385) Polysensitized individuals % (N=173)
Scalp	15.8% (62)	8.0% (15)	21.2% (45)	20.2% (35)
Periorbital region	12.5% (49)	13.9% (26)	23.6% (50)	17.3% (30)
Periauricular region	12.0% (47)	11.8% (22)	16.0% (34)	11.6% (20)
Perioral region	7.1% (28)	8.0% (15)	11.3% (24)	11.6% (20)
Remaining part of face	13.0% (51)	14.4% (27)	17.5% (37)	20.2% (35)
Neck	12.0% (47)	12.8% (24)	19.8% (42)	24.3% (42)
Shoulders	6.4% (25)	6.4% (12)	10.4% (22)	11.0% (19)
Armpits	9.4% (37)	12.8% (24)	15.6% (33)	20.8% (36)
Cubital folds	8.9% (35)	8.6% (16)	34.4% (73)	41.0% (71)
Arms	14.2% (56)	12.8% (24)	20.3% (43)	24.3% (42)
Hands/wrists	61.1% (240)	71.7% (134)	59.4% (126)	68.2% (118)
Chest	9.2% (36)	10.7% (20)	15.1% (32)	16.2% (28)
Stomach	9.9% (39)	14.4% (27)	20.3% (43)	17.9% (31)
Back	10.2% (40)	11.2% (21)	15.6% (33)	25.4% (44)
Buttocks	6.4% (25)	5.9% (11)	9.9% (21)	16.2% (28)
Popliteal folds	7.6% (30)	5.9% (11)	31.6% (67)	37.0% (64)
Legs	18.3% (72)	11.2% (21)	22.6% (48)	26.6% (46)
Feet/ankles	21.9% (86)	22.5% (42)	28.8% (61)	30.1% (52)
Anogenital region	6.1% (24)	4.3% (8)	9.4% (20)	9.2% (16)

TABLE 16: *Distribution of dermatitis on 19 different skin sites at time of debut*

EXPLANATORY VARIABLES	total population *		NO ATOPIC ECZEMA *		ATOPIC ECZEMA *	
	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)
No atopic eczema	542	REFERENCE	-	-	-	-
Atopic eczema	354	**1.43 (1.06-1.93)**	-	-	-	-
Educational level						
< 10 yrs	108	REFERENCE	64	REFERENCE	42	REFERENCE
11-12 yrs	344	0.70 (0.44-1.11)	203	0.55 (0.30-1.01)	139	0.97 (0.47-2.00)
13-14 yrs	106	0.93 (0.53-1.64)	74	0.69 (0.33-1.42)	31	1.09 (0.41-2.87)
15-16 yrs	202	0.69 (0.42-1.14)	110	0.51 (0.26-1.01)	90	1.03 (0.47-2.22)
> 17 yrs	81	0.68 (0.37-1.28)	58	0.50 (0.22-1.10)	23	1.01 (0.35-2.92)
Duration of disease						
0-9 yrs	191	REFERENCE	150	REFERENCE	36	REFERENCE
10-19 yrs	208	**2.20 (1.40-3.47)**	150	**2.20 (1.28-3.76)**	57	2.10 (0.84-5.30)
20-29 yrs	157	**2.54 (1.56-4.12)**	87	**3.34 (1.82-6.14)**	70	1.79 (0.74-4.35)
30-39 yrs	156	**2.54 (1.56-4.15)**	81	**2.19 (1.16-4.13)**	74	**2.84 (1.16-6.91)**
>= 40 yrs	192	**2.72 (1.67-4.42)**	74	**2.87 (1.49-5.53)**	117	2.29 (0.89-5.33)
Outbreak frequency						
Persistent eczema	223	REFERENCE	112	REFERENCE	111	REFERENCE
Dermatitis > ½ of period	225	0.83 (0.56-1.23)	140	1.32 (0.76-2.30)	79	**0.54 (0.30-0.99)**
Dermatitis = ½ of period	132	0.75 (0.48-1.19)	75	0.92 (0.47-1.80)	57	0.68 (0.35-1.33)
Dermatitis < ½ of period	297	0.79 (0.55-1.15)	194	1.43 (0.84-2.42)	102	**0.43 (0.25-0.77)**
No leg ulcers	833	REFERENCE	504	REFERENCE	321	REFERENCE
Leg ulcers	71	1.13 (0.68-1.90)	38	1.08 (0.52-2.24)	33	1.05 (0.49-2.28)

OR = odds ratio, CI = confidence interval * adjusted for sex, age, and patch test year

TABLE 17: *Associations between atopic eczema, educational level, duration of disease, outbreak frequency, leg ulcers and polysensitization assessed by logistic regression analyses. Three analyses are presented based on a population of all respondents, and patients with and without atopic eczema. Significant associations are in* ***bold****.*

Skin sites affected with dermatitis at debut are illustrated in TABLE 16. Hands and wrists was the most frequent and the anogenital region was the least frequent skin area affected for both polysensitized and single/double-sensitized individuals with and without AE.

4.3.5. Associations and polysensitization

Results from the multivariate regression analyses are given in TABLE 17 and TABLE 18. AE was significantly positively associated with polysensitization (OR 1.43, 95%CI 1.06-1.93). Duration of disease was significantly associated with polysensitization in the total respondent population (ORs 2.20-2.72) and in the population of patients without AE (ORs 2.20-2.87). Outbreak frequency was significantly associated with polysensitization in the AE group. Dermatitis on the hands/wrists, in the armpits and on the back was positively associated with polysensitization with ORs 1.58 (95%CI 1.18-2.11), 1.56 (95%CI 1.02-2.37) and 1.91 (95%CI 1.16-3.14), respectively. Dermatitis on the scalp was negatively associated with polysensitization (OR 0.66, 95%CI 0.44-0.99). In the group of patients without AE, dermatitis on the scalp (OR 0.42, 95%CI 0.22-0.82) and on the legs (OR 0.50, 95%CI 0.27-0.95) showed a negative association with polysensitization, and dermatitis on the hands/wrists (OR 1.63, 95%CI 1.09-2.43) showed a positive association with polysensitization. In the group of patients with AE, dermatitis on the hands/wrists (OR 1.63, 95%CI 1.02-2.61) and on the back (OR 2.84, 95%CI 1.38-5.84) at time of debut was positively associated with polysensitization and dermatitis on the stomach (0.40, 95%CI 0.19-0.83) was negatively associated with polysensitization.

EXPLANATORY VARIABLES	total population † No. 965		NO ATOPIC ECZEMA * No. 580		ATOPIC ECZEMA * No. 385	
	OR	95% CI	OR	95% CI	OR	95% CI
Scalp	**0.66**	**0.44-0.99**	**0.42**	**0.22-0.82**	0.90	0.50-1.63
Periorbital region	0.85	0.56-1.30	1.38	0.87-2.44	0.55	0.29-1.04
Periauricular region	0.88	0.56-1.37	1.06	0.58-1.91	0.62	0.30-1.29
Perioral region	1.13	0.68-1.90	1.11	0.54-2.30	1.37	0.63-2.96
Remaining part of face	1.20	0.80-1.79	1.10	0.64-1.91	1.27	0.67-2.41
Neck	1.14	0.74-1.74	0.92	0.49-1.71	1.36	0.74-2.53
Shoulders	0.62	0.33-1.18	1.03	0.39-2.75	0.43	0.17-1.05
Armpits	**1.56**	**1.02-2.37**	1.75	0.95-3.20	1.51	0.82-2.77
Cubital folds	1.22	0.80-1.88	1.19	0.56-2.52	1.32	0.74-2.35
Arms	1.10	0.72-1.68	1.09	0.57-2.09	1.27	0.69-2.32
Hands/wrists	**1.58**	**1.18-2.11**	**1.63**	**1.09-2.43**	**1.63**	**1.02-2.61**
Chest	0.99	0.59-1.65	1.13	0.52-2.45	0.94	0.44-2.02
Stomach	0.86	0.54-1.38	1.55	0.81-2.96	**0.40**	**0.19-0.83**
Back	**1.91**	**1.16-3.14**	1.43	0.64-3.19	**2.84**	**1.38-5.84**
Buttocks	1.40	0.78-2.49	0.87	0.33-2.28	1.92	0.86-4.30
Popliteal folds	0.93	0.58-1.48	0.70	0.30-1.64	1.05	0.57-1.94
Legs	0.72	0.48-1.09	**0.50**	**0.27-0.95**	1.05	0.58-1.89
Feet/ankles	0.93	0.67-1.29	1.01	0.65-1.58	0.78	0.47-1.31
Anogenital region	0.81	0.46-1.43	0.77	0.32-1.87	0.88	0.39-1.97

* adjusted for sex, age and patch test year
† adjusted for sex, age, patch test year and atopic eczema
OR = odds ratio, CI = confidence interval

TABLE 18: *Associations between dermatitis on specified skin areas at time of debut and polysensitization based on logistic regression analyses for the total population examined and two subpopulations with and without atopic eczema, respectively. Significant associations are presented in* ***bold****.*

A

ATOPIC ECZEMA

SKIN AREA	Scalp	Periorbital region	Periauricular region	Perioral region	Remaining part of face	Neck	Shoulders	Armpits	Cubital folds	Arms	Hands / wrists	Chest	Stomach	Back	Buttocks	Popliteal folds	Legs	Feet / ankles	Anogenital region
Scalp																			
Periorbital region	3,31																		
Periauricular region	7,03	7,76																	
Perioral region		11,0	5,87																
Remaining part of face	3,47	6,64	5,21	6,53															
Neck	3,77	4,06	5,29	5,62	6,49														
Shoulders					4,74	7,55													
Armpits						4,39													
Cubital folds								3,26											
Arms					2,86	2,88	10,8												
Hands/wrists																			
Chest	3,20	3,20			5,59	4,59	13,8	3,77		7,70									
Stomach				3,52		4,10	8,30	3,38		5,48		12,5							
Back					3,43	3,50	28,1			6,83		6,48	10,4						
Buttocks	3,52					3,60	10,3			5,88		11,3	13,2	10,9					
Popliteal folds								5,02	22,3						3,02				
Legs							10,4			6,19		4,85	6,05	9,43	7,46				
Feet/ankles										3,15	3,00		2,68	3,25	3,57		3,63		
Anogenital region	4,06												4,58		5,71				

B

NO ATOPIC ECZEMA

SKIN AREA	Scalp	Periorbital region	Periauricular region	Perioral region	Remaining part of face	Neck	Shoulders	Armpits	Cubital folds	Arms	Hands / wrists	Chest	Stomach	Back	Buttocks	Popliteal folds	Legs	Feet / ankles	Anogential region
Scalp																			
Periorbital region	3,40																		
Periauricular region	4,23	4,41																	
Perioral region		5,40	4,23																
Remaining part of face		3,06		4,03															
Neck		3,54		5,19	3,96														
Shoulders						11,3													
Armpits																			
Cubital folds	4,82							5,51											
Arms						4,87	21,4		4,11										
Hands/wrists																			
Chest	3,37					6,27	19,1			5,20									
Stomach						4,33	8,57			3,93		14,1							
Back	3,26					4,93	29,3			6,98		28,4	11,8						
Buttocks							27,6			10,1		10,1	13,5	23,6					
Popliteal folds								5,43	20,4										
Legs							7,58			12,1		3,77	3,39	7,01	4,85				
Feet/ankles																			
Anogenital region																			

Numbers represent odds ratios (ORs). The grey-toned squares represent the quartile of ORs with highest values.

FIGURE 12: *Associations between pairs of skin sites affected with dermatitis at time of debut.*

4.3.6. Associations between dermatitis on different skin sites

FIGURE 12 illustrates associations between dermatitis on different skin sites for patients with and without AE. A higher number of associations were found in the AE group. Skin sites related to the head were significantly associated as were skin sites on the trunk. Dermatitis in cubital and popliteal folds was significantly associated with each other. Dermatitis on the hands was mostly an isolated finding. Dermatitis on the feet/ankles and in the anogenital region were also isolated locations for individuals without AE.

4.3.7. Patch test readings and patients with atopic eczema

Patients with AE had a higher frequency of irritant reactions (IRs) for potassium dichromate readings on D3 and on D7 compared with patients without AE (results not shown) but not for any other IR reading for the remaining 22 allergens. Patients with AE had a higher frequency of +? reactions for neomycin on D3, benzocaine on D2 and on D3, formaldehyde on D2, mercapto mix on D3, methylchloroisothiazolinone/methylisothiazolinone on D7, MBT on D3, and for SL mix on D7 readings (results not shown) and a higher frequency of follicular reactions for PPD on D3, quaternium-15 on D7, and SL mix on D2 readings. No differences in frequency of 1+, 2+ or 3+ positive reactions were detected between patients with and without AE.

5. DISCUSSION

5.1 GENERAL DISCUSSION

5.1.1. Defining polysensitization

A definition of polysensitization as ≥ 3 contact allergies was used throughout the study parts to reduce the number of patients where allergen combinations could be explained exclusively by cross-reactivity. The individual combinations of allergens in patients with 3 contact allergies often appear randomly [1;4]. Combinations of 2 allergens, contrary to triplet combinations, more often appear in a non-random pattern caused by cross-reactions or associated exposure [29-33]. Only very few specific triplet allergen clusters occur [4;5;34-37]. When keeping the definition of polysensitization at 3 or more contact allergies compared with 2 or more contact allergies, the chance of 2 out of 3 allergens cross-reacting may be seen, but the chance of 3 out of 3 allergens cross-reacting is considered to be minor. The definition minimizes the possibility of *all* allergens in the individual combinations being cross-reactions. We acknowledge that the definition of polysensitization is crude and arbitrary and cannot entirely eliminate patients where cross-reactivity explains the development of polysensitization. The definition of polysensitization as 3 or more contact allergies seems reasonable when focusing on patients with true independent multiple sensitizations.

5.1.2. Angry back and polysensitization

Angry back in its original definition is not regarded as a major contributor to false multiple reactions. When the angry back phenomenon was first described, no experimental data was presented to support this observation [25]. Lack of persistence and lack of relevance of patch test reactions were taken as evidence of the occurrence of the angry back / excited skin syndrome [26;28;70;71]. Several studies have attempted to reproduce the angry back phenomenon, but the arrangement of the patches on the back has repeatedly been shown not to have any enhancing effect on their neighbour patch test reactions or on more distant patch test reactions [72-75] and the phenomenon could not be reproduced in patients formerly proclaimed to have angry back reactions [76]. The angry back and excited skin syndrome should be distinguished from unspecific exacerbation of dermatitis over the entire patch test area, which can be provoked by patch testing. This phenomenon was not included in the original description [25;28].

5.1.3. EBS as reference series for determining sensitization status

In manuscript I, II, IV and V, the definition of polysensitization was based on ≥ 3 contact allergies to allergens in the *European Baseline Series (EBS)* excluding possible reactions to additional series. It can be argued that some of the patients with 1-2 contact allergies were misclassified. They could have reacted to allergens in additional patch test series which were not counted due to the chosen delimitation. In five European dermatology centres, 5-23% of patients tested reacted only to allergens in series other than the EBS, and 77-95% of patients with positive reactions reacted to allergens in the EBS where some also reacted to allergens in additional series [77]. Some allergies will be missed by counting only reactions to the EBS, but the EBS does detect the majority of allergies. Additionally, all patients were tested with the EBS but not necessarily with additional series or with the same additional series. Including reactions to allergens in additional series would introduce a selection bias. In the EBS, triplet clusters can be

seen together because of associated exposures but not because of cross-reactivity except from IPPD, which may cross-react with PPD and benzocaine. If reactions to allergens in additional series are included the possibility of cross-reactions will also increase, hence the considerations concerning the definition of polysensitization.

In the second manuscript, reactions to MBT and quaternium-15 were excluded from all counts of additional allergies. MBT is a constituent in mercapto mix and quaternium-15 is a formaldehyde-releasing substance. Reactions to MBT and to mercapto mix or to formaldehyde and quaternium-15 may be a reaction to the same allergen and may not represent two distinct allergies. The procedure reduced the risk of duplicate counts of the same allergy. It could be argued that this should have been done in the other manuscripts too.

Reactions to allergens in a supplementary standard series were included in manuscript III. All study subjects had been tested with this additional series. Only a limited number of patients were included and it was practically possible to assess every individual combination of allergens and exclude combinations of chemically / structurally identical allergens.

Some allergens changed concentration and composition during the 20-year period and were not evaluated separately. Elicitation of a contact allergy is dose-dependent [42] and the intrinsic properties with reference to potency and irritant potential may vary between the substituted allergens. Such changes can influence the outcome of patch testing and can represent a bias in data which is non-differential.

5.1.4. Choice of reference group

Throughout the study parts, patients with 2 allergies were joined with patients with 1 allergy into one reference group and examined against patients with ≥ 3 contact allergies. It could be argued that such a binary model simplifies the results and that nominal and ordinal models would have contributed with more detailed information. The phenomenon of increased inherent susceptibility attributed to patients with multiple contact allergies was also graded according to number of contact allergies [1]. For simplicity, a reference group was initially chosen where 1 and 2 contact allergies were combined. An ordinal design would likely have contributed with more detailed information, and we acknowledge that the chosen binary model inherently simplifies the results.

Patients referred to patch testing but with negative results were excluded from manuscript II-V. These patients constitute a heterogeneous group of endogenous eczemas and irritant contact dermatitis and also non-dermatitis dermatoses undergoing diagnostic elucidation [78]. The aim was to examine polysensitized patients against other groups within the field of contact allergies and not within the field of eczemas. Patients without recorded allergies were, therefore, omitted from the studies.

5.2. STUDY PART I – THE DATABASE STUDY

5.2.1. Result-specific discussion

5.2.1.1. Prevalence

The prevalence of patients with multiple contact allergies was 5.1% and correlates well with previously reported prevalences for multiple contact allergies [1;4;5]. Regulatory interventions in Denmark have effectively decreased the sensitivity rate for nickel and chromate allergy [79]. Increasing and decreasing trends in sensitivity rates for other allergens have also been noticed [79-83] and conform to similar trends identified in other populations [84-88]. Despite this, the prevalence of polysensitization remained stable during the 20 years studied indicating that the propensity to develop multiple contact allergies is independent of type of allergen. Frequency of polysensitization over time has not been estimated in other studies.

Underlying time trends in population demographics occurred during the 20 years studied. The age median increased by 5 years, and the fraction of women tested also increased somewhat in accordance with one other report [89]. A slight increase in overall sensitivity rate and in sensitivity rates for multiple-allergic patients could be expected [90;91]. This was not the case and could mask a small decrease in sensitivity rates. The number of patients patch tested each year also increased. Assuming unchanged sensitivity rates in the background population, such liberal referral practice should dilute the sensitivity rates. Since this was not the case either; an increase in sensitivity rates in the referred population could be the case, or one could state that a more liberal patch test reading had taken place during the years. In contrast, the effect of increase in total number of patients tested could be outbalanced by the effect of increase in age median and female sex.

5.2.1.2. Demographics & additional patch tests

Four out of 5 patients with multiple contact allergies were women. Epidemiological studies report a high prevalence of contact allergies among women [33;92;93]. In one study, the female sex reacted more strongly on challenge than men [94]. A higher induction rate for women [95] was also noted in one study but could not be confirmed in two other studies [96;97]. The dominance of women among patients with multiple contact allergies can be explained by sex-specific behaviour with increased exposure and female dominance in occupations and domestic work with high exposure to irritants and allergens [90].

The risk of polysensitization increased with age and the effect of age differed between the sexes. Other epidemiological studies state both a decreasing [92;93] and an increasing risk [91] of contact allergies with age and no correlation between age and number of contact allergies [21]. Sensitivity rates for specific allergens show both upward and downward trends with increasing age [33;98]. With increasing age, the opportunity and duration of exposure to environmental allergens logically increases and should logically also increase the prevalence rates. If genetics were the main causal factor of development of multiple contact allergies, this subgroup would be expected to be younger than observed. Elderly individuals also react more slowly and less intensely at challenge [99] compared with young individuals. Induction of new contact allergies seems impaired [100] in elderly individuals in one study but unchanged for elderly women in another study [97]. Elderly men reacted more slowly to induction but eventually reached the same

degree of sensitization as younger men [97]. High concentrations of DNCB sensitize the same number of elderly and young individuals but elderly individuals react less to intermediate doses [101]. Cumulative environmental exposure seems to be an important causal factor in development of multiple contact allergies.

90% of multiple-allergic patients had already developed multiple sensitizations when referred to the hospital sector, excluding the opportunity of primary prevention at this stage. A larger part of patients with multiple contact allergies were patch tested multiple times compared with patients with 1-2 contact allergies but the difference was relatively small. Patients tested several times typically have either recurrent or persistent dermatitis, which raises the suspicion of new allergies. Duration of disease has been associated with number of contact allergies in leg ulcer patients [11-13]. Indeed patients tested multiple times had an increased risk of having additional contact allergies.

5.2.1.3. Allergens and polysensitization

Patients with multiple contact allergies seem approximately equally susceptible to all substances in the EBS of equal sensitizing capacity. Ubiquitous and potent allergens were the most frequent sensitizers and the non-ubiquitous and weak allergens were uncommon sensitizers, as would be expected due to exposure pattern, allergen availability and allergen potency. Any increased susceptibility to sensitization among patients with multiple contact allergies does not seem to be directed towards specific chemical configurations, which is also concluded in one other study [1]. Contrary, Landsteiner et al reported a selective direction in susceptibility [102] and certain genetic markers are associated with specific allergens only [2;47].

Twelve allergens showed statistically significant associations with polysensitization. Parabens mix has previously been shown to be positively associated with polysensitization [38]. Mercapto mix occurs more frequently in association with other allergens than as a single allergy [4] but did not reach statistical significance in this study. Nickel sulphate has formerly been shown not to be associated with polysensitization [4;5]. The risk of neomycin allergy increased with additional allergies [39] but neomycin allergy in itself did not increase the risk of polysensitization in our study. Myroxylon pereirae, fragrance mix and formaldehyde often occur together with additional allergies [20;22], which could be confirmed only for Myroxylon pereirae. A positive association between an allergen and polysensitization does not mean that the majority of polysensitized individuals acquire allergies to these allergens, hence the absolute sensitivity rates. For the allergens with the strongest associations, about every second individual with these particular allergies has ≥ 2 additional allergies.

The allergens with positive and negative associations with polysensitization, respectively, have highly variable chemical structures, allergen potency, typical exposure settings and availability. Allergens known to cross-react also displayed opposite signs in their association with polysensitization. The only possible interpretation of the results is the presence or absence of associated exposure with other allergens in the baseline series. The allergens with significant positive associations often occur in settings with multiple allergen exposure and are often more difficult to identify and avoid. The allergens with significant negative associations are often found in iso-

lated exposure settings and can more easily be identified and avoided. The limitation of this interpretation is that the explanation does not apply to the allergens without significant associations. It is not known if the allergens were primary or secondary allergies.

5.2.2. Methodological considerations

5.2.2.1. Database

The strength of the Danish Database lies in the large population size and the 100% degree coverage, which eliminates any selection bias. Almost all patients are tested with the entire set of allergens in the EBS, creating a high degree of completeness to the data. Only one centre contributed to the data pool, which eliminates any inherent biases in multi-centre designs. Nevertheless, a single centre can effectively decrease generalisation of the results. The study population was, however, representative compared with other European units regarding the overall sensitivity rate, the rate of multiple contact allergies and allergen-specific prevalences, and age and sex composition [1;5;103;104].

Other important data for the assessment of representativeness of the cohort are assembled in the MOAHLFA index (M = male, O = occupation, A = atopy, L = leg dermatitis, H = hand dermatitis, F = face dermatitis, A = age) [105]. It would be preferable if all factors in the MOAHLFA index had been reported for the total study population. Unfortunately, information about the remaining factors (occupation, atopy, leg / hand and face dermatitis) in the MOAHLFA index was not obtainable for the entire period. Such information has been systematically collected and registered in the database only since 1994.

5.2.2.2. Statistics

The effect of the confounding variables age and sex was accounted for in the logistic analyses performed. Exposure to allergens is not equal over different age strata due to differences in lifestyle factors and generally because of increased exposure time [98]. Other confounding effects for the association between allergens and polysensitization may be the remaining factors in the MOAHLFA index that could not be assessed. They may differ between cases and controls.

It should be emphasized that the odds ratio estimates in manuscript II are not directly comparable since one analysis was made for each allergen. The analyses of association between allergens and polysensitization were not adjusted for multiple testing. The significance level could be affected by such an adjustment and creates a statistical limitation.

5.2.2.3. Patch test procedure

No patch test system has a 100% reproducibility in right-left comparisons [106-109] including the Finn Chamber technique. Several factors can affect the outcome of patch testing if not accounted for. The sources of variability include the patch test technique and materials (type of patch test system [109], type of vehicle [41], concentration of allergen [42], amount of material applied [110], sources of allergens [111], occlusion time [112], reading times [113], and intra- and interobserver variability in interpretation of response and in preparation of material [114;115]); biological factors (active dermatitis at the test site, inter- and intraregional variations in skin responsiveness

[62;116;117], intra-individual variation [118]); and environmental factors (seasonal changes [119], simultaneous medication [120;121] and sun exposure [122]).

Adjustable factors were standardised to minimize the risk of inconsistencies in methodology. The patch test system and source of patch test allergens, preparations, application technique and site, materials, occlusion time used, defined reading times and standardized interpretations were unchanged during 1985-2005. Furthermore, the patch testing and readings were performed by a very small number of experienced and specially trained personnel. In addition, D7 readings were performed routinely for all patients and for all allergens, supplementing D2 and D3/4 readings, optimizing the detection of contact allergies to allergens known to give reactions on D7 e.g. neomycin [113]. Patch testing is routinely postponed in the case of active dermatitis on the back at test time, recent UV exposure, systemic or topical corticosteroid use or if an exacerbation of dermatitis occurs during patch testing. This procedure minimizes false positive and false negative reactions. Observations of multiple positive reactions separated by normal appearing skin were generally taken as genuine multiple reactions and these patients were not retested by standard. We cannot exclude that apparently normal but recently inflamed skin on the back still has enhanced skin reactivity [62] and could have caused false positive reactions in some patients; however, it is considered a minor and non-differential problem.

Patch testing is performed throughout the year and seasonal influences may have affected the outcome of patch testing. Meteorological influences were, however, restricted to a few allergens in a recent study [119]. The intra-individual variation in patch test reactivity is great, sometimes even resulting in negative test results with positive test results to the same allergen at earlier and later testing [118]. It can obviously not be controlled.

5.2.2.4. Defining positive reactions

Readings of “Not Tested: Sensitized” (NT:S) were classified as positive reactions. When NT:S is registered an active evaluation is made as to whether a true sensitization has previously occurred. Some registrations of NT:S may have been based on patient history and clinical picture exclusively and may not represent true positive reactions. Such bias is minor since only 0.0-2.2% of all patch test readings included NT:S-registrations; furthermore, it is non-differential.

5.3. STUDY PART II – THE DOSE-RESPONSE STUDY

5.3.1. Result-specific discussion

Polysensitized individuals did not show increased reactivity in the elicitation phase of the allergic response expressed as unique elicitation dose-response curves for either each of the allergens separately or for the dose-response curves combined. The dose-response curves were merged for all three allergens to increase the total number of test subjects and power. The choice to combine individuals with 1 and 2 contact allergies in the reference group could have obliterated any true but small difference. The reactivity may be a graded phenomenon which increases with increasing number of contact allergies [1].

The circumstances surrounding sensitization are not standardised. Test subjects consequently expressed heterogeneity regarding real-life conditions of exposures leading to sensitization. The elicitation response is dependent on the induction dose: the greater the induction dose, the greater the elicitation response [42]. Heterogeneity between cases and controls regarding exposure conditions leading to sensitization may have influenced strength and threshold of the elicitation response, explaining the lack of difference observed. In a similar study, induction was controlled and differences in induction and elicitation dose-response curves could be visualized [1]. Allergic mechanisms are allergen specific, which also may explain the different outcomes when using MDBGN, PPD and nickel sulphate in contrast to DNCB [1].

Only a subgroup of polysensitized individuals without intense exposure as cause of polysensitization expresses an increased sensitivity [1;42]. Heterogeneity, not only between cases and controls regarding conditions of exposures but also within our case group according to specific conditions leading to polysensitization, may also explain the lack of difference observed.

Active inflammation, topical or systemic treatments did not affect the results as none of the test subjects had dermatitis at time of patch testing or were treated with topical steroids or systemic immunosuppressive medications within 1 week and 2 weeks, respectively, of patch testing. Seasonal variations were unlikely to have affected the results since polysensitized and single/double-sensitized individuals were tested randomly, and not in groups, during periods of up to 6 months. Neither was there any age or sex difference which could have affected the results. Although, four persons in the PPD arm were exposed to UV light on their backs during the 3 weeks before patch testing, this was not during the 7 days before patch testing. They were all in the group with 1-2 contact allergies. One person in the MDBGN arm had the patches removed after 24 hours. Both factors could have affected the elicitation thresholds for these five test subjects [42;112]. If so, they would have contributed to greater differences rather than unity between the two test groups.

5.3.2. Methodological considerations

The dose-response study consists of data compiled from 3 original studies. It was not conducted with the primary intention to study the elicitation response in polysensitized individuals versus individuals with 1-2 contact allergies. This creates a great weakness of the study and the results should be regarded as exploratory.

The main differences in methodological design between the 3 studies consist of non-identical reading scales and readings being performed blinded for nickel sulphate and MDBGN but not for PPD. Choice of reading scale and the non-blinded design in the PPD arm may have affected threshold determination. However, the conditions for determination of reactivity were identical within each dose-response arm. Therefore, the differences in methodological design were not relevant for the estimation of relative sensitivity.

The dose-response relations were based on data from small test groups, which reduces the precision of the calculated relative sensitivities illustrated by the wide CI 95%s. The polysensitized test group was particularly small. The statistical calculations were also performed for a

monosensitized group versus a group with ≥ 2 contact allergies. This grouping strategy is more optimal from a statistical point of view. It almost equalized the number of test subjects in each study group but it did not change the results nor increase the precision of results. The differences between the single/double- and polysensitized group were still non-significant. It is possible that a difference in relative sensitivity (inherent or acquired susceptibility) between the two groups truly exists, but the power of the study was inadequate to establish any difference.

Based on the aforementioned limitations in methodological design and test size, it must be emphasized that the results need to be confirmed in studies designed and planned with the specific aim and hypothesis in mind.

5.4. STUDY PART III – THE QUESTIONNAIRE STUDY

5.4.1. Result-specific discussion

5.4.1.1. Atopic eczema and polysensitization

The prevalence estimate of atopic eczema (AE) reached 36% in this study, but it corresponds well with prevalences of AE in other patch test populations ranging from 16.7% to 46% [5;123-125]. Nearly half of our polysensitized patients (45%) and 31% of the patients with 1-2 contact allergies had AE and patients with AE had an increased risk of polysensitization compared with non-AE patients. In a recent German study, 38.4% of all polysensitized individuals and 37.8% of single/double-sensitized individuals had AE [125].

Earlier experimental studies reported a reduced ability to develop contact allergies and a diminished response at challenge among patients with AE [126-129]. The ability to develop contact allergies was inversely related to severity of atopic disease and level of altered leucocyte function [126;129-131]. In support, decreased sensitivity rates among AE patients compared with non-AE patients was also reported [132;133]. In the recent years, there has been consistency in clinical studies, showing that contact allergies are frequent events among patients with AE; 17.1%-54% of patients with AE have contact allergies [124;125;134-136] and around $^{3}/_{10}$ of contact-allergic AE patients have 2 or more contact allergies [125;135]. Contact allergies seem to be equally prevalent among individuals with and without AE [125;134;137]. Frequent and persistent exposure to various treatments containing multiple substances and impaired skin barrier with increased allergen absorption in AE patients may outbalance any reduced ability for sensitization. This is supported by a positive relationship between severity and duration of AE and frequency of contact sensitizations [138;139] and a higher sensitivity rate among non-healed compared with healed patients with AE [140].

It can be argued that individuals with AE have a higher skin reactivity [141-144] and more easily develop irritant and doubtful patch test reactions. Frequent irritant and doubtful reactions hold the potential for misinterpretations of patch test reactions as false positive reactions. Such increased hyperreactivity was present both in clinically normal and dry skin of patients with AE in one study [143] and in another study seemed present only in patients with active AE and not among patients with a history of AE but no active lesions [141]. Acute skin irritation responses are not always more pronounced in patients with AE [145]. The significant differences in frequencies of

irritant reactions, follicular and doubtful reactions between AE and non-AE patients in this study occurred in a non-systematic way and did not result in a generally higher rate of positive readings in patients with AE. Two other studies also assessed the pattern of patch test reactions and did not find any great differences between patients with and without AE [124;134].

AE is associated with filaggrin loss-of-function mutations [146] and these mutations are also suspected to be associated with contact allergy [2;52]. It is possible that our finding regarding AE and polysensitization is confounded by filaggrin mutations.

5.4.1.2. Leg ulcers, leg dermatitis and polysensitization

The prevalence of leg *ulcers* in polysensitized patients reached 8.6%. Leg ulcers were not overrepresented in polysensitized patients and did not constitute a risk factor for polysensitization. In contrast, leg *dermatitis* was negatively associated with polysensitization in patients without AE when compared with dermatitis on other skin sites. Leg dermatitis is more common in patients without AE compared with patients with AE [125;134]. New topical treatments with low sensitizing potency developed in the recent years may have diluted the previously seen high frequency of polysensitization among leg ulcer and stasis dermatitis patients, as seen in one recent study [147]. A further indication of this is the stagnation in recent years of the frequency of leg dermatitis in a patch test population, despite increasing mean age [89]. The higher frequency of AE in the polysensitized group may also have diluted any difference in leg *ulcer* frequency between the poly- and single/double-sensitized group, but leg ulcers remained un-associated with polysensitization in multivariate analyses with adjustment for AE. The risk of polysensitization correlates with duration of leg ulcers and stasis dermatitis [11-13] which was also adjusted for in the multivariate analyses. Leg *dermatitis* covered dermatitis anywhere on the leg including the thigh and lower leg. The variable does not exclusively represent stasis dermatitis.

5.4.1.3. Educational level and polysensitization

The educational level did not show any different pattern for polysensitized compared with single/double-sensitized patients. In general, patients with lower educational level have a higher risk for contact allergy [136]. The educational level reflects skills and qualifications obtained from school and vocational education. Skills acquired by type of work, experience or informal training were not assessed.

5.4.1.4. Clinical characteristics

a. Duration and course of disease

Polysensitized individuals have a long duration of disease, respectively 22 and 33 years, dependant on whether they have AE. The influence of contact allergies on duration and course of disease differed between the groups of patients with and without AE.

The *duration of disease* corresponds to the length of disease in years regardless of outbreak frequency. Contact allergies did not influence the duration of dermatitis for patients with AE. Both single/double-sensitized and polysensitized AE individuals had long duration of eczema. The duration was not significantly different between the two groups consistent with one other study where the course of AE was not impaired by contact allergies [135]. Other studies report

long duration of disease among patients with AE [148-151]. Polysensitized patients without AE had longer duration of dermatitis than did single/double-sensitized individuals without AE. Contact allergy has previously been shown to influence the prognosis of dermatitis negatively in a hand dermatitis population [65;66]. Low age at onset of dermatitis can be important for long-term persistency of symptoms [65]. Age at onset did not diverge and diverged only minimally between the poly- and single/double-sensitized groups with and without AE, respectively. The absolute difference was small and does not explain the difference in duration of disease.

Persistent and intermittently occurring dermatitis corresponds to the outbreak frequency. Nearly 40% of polysensitized AE individuals had persistent dermatitis compared with about 25% of the single/double-sensitized AE group. The rest had intermittently occurring dermatitis. About 20% of both polysensitized and single/double-sensitized non-AE patients had persistent dermatitis and the rest had intermittent dermatitis. In one former study, no difference between formaldehyde-allergic patients with and without additional allergies was found with respect to frequency of dermatitis eruptions [22].

The positive association between duration of disease and number of contact allergies may reflect that long duration of skin disease with impaired skin barrier predisposes to polysensitization, as is seen for leg ulcers [11-13], or reflects difficulty in proper avoidance of contact with the relevant allergens due to the many allergies with multiple exposure routes. A negative correlation between number of contact allergies and improvement of allergic contact dermatitis has been noticed [21]. The course of dermatitis correlates with the ability to comply with instructions and with understanding of diagnosis [22;152;153]. The ability to comply with instructions and avoid allergens is dependent on educational level, type of allergy, sex, ethnic background, family circumstances and age [152;154;155]. Whether the long duration of disease is the cause or consequence of polysensitization cannot be answered in this retrospective design.

b. Site of dermatitis

The hands were the most common site of dermatitis regardless of AE and sensitization status (~64%). Previous reports of hand dermatitis reached 26-59% in patch test populations, which also included patients with negative patch tests [5;104;134] and hand dermatitis is also particularly common among adults with AE [123;125;134]. Patients with hand dermatitis had an increased risk of polysensitization, and dermatitis on the hands was the only skin site that maintained the association with polysensitization regardless of AE status. One study reported a lower frequency of hand dermatitis in polysensitized patients (26%) than in monosensitized individuals (44.4%), which could not be confirmed in this study [20]. The hands were associated with the greatest number of contact allergies in another patch test population compared with other skin sites [156].

The skin of the hands is frequently exposed to irritants and allergens but development of multiple contact allergies may, however, also cause dermatitis on the hands. The time/cause-relationship cannot be further elucidated with the chosen design. Hand dermatitis was not associated with dermatitis in other skin areas (except the feet in the AE group).

Dermatitis on other skin sites was significantly associated with polysensitization but was not as consistent as hand dermatitis. Scalp dermatitis was negatively associated with polysensitization primarily in patients without AE. The majority of patients with scalp dermatitis suffer from endogenous eczema such as seborrhoeic dermatitis [157]. Axillary dermatitis was associated with polysensitization but lost its association in the subgroups according to AE status, probably because of lesser power. The semi-occlusive nature of the armpits and shaving may increase skin absorption [158]. Deodorants, as the typically consumer product applied to this area, contain multitudes of chemicals, including irritants, which can reduce the induction threshold [41]. All factors may contribute to the observed association. Dermatitis on the back and on the stomach was, respectively, positively and negatively associated with polysensitization in patients with AE. The many positive patch test reactions on the back in polysensitized patients during patch testing may lead the study subjects to mark the back as affected at time of debut. Patients with AE also often show irritant reactions when patch tested [124;139] and the combination of many irritant and allergic reactions, or more pronounced reactions because of hyperreactivity [141-144;159], may also lead to a false demarcation. Exacerbation of diffuse dermatitis on the back provoked by patch testing may also explain the observed association. Dermatitis on the stomach could be a remnant of nickel allergy with nickel in trouser buttons [156]. Nickel allergy is predominantly an isolated allergy as found in study part I.

c. Extent of dermatitis

The extent of dermatitis at debut was not more pronounced in polysensitized patients in contrast to one previous study [20]. The extent of dermatitis is determined by exposure, which is related to the type of allergy and allergen availability and it is not a result of number of sensitizations. If polysensitized individuals have an increased reactivity [1], it may result in more severe dermatitis but not necessarily more widespread dermatitis.

5.4.2. Methodological considerations

5.4.2.1. Selection bias

All polysensitized individuals from the database still alive and living in Denmark at the time of study execution were included. This procedure noticeable reduces any bias in case selection. There is no reason to believe that patients lost to follow-up owing to death bias results. Patients lost to follow-up because of emigrations may be related to factors concerning dermatitis. Only 9 out of 759 patients had emigrated.

The controls were drawn from the same hospital population as cases. Both groups have been subjected to the same selection factors leading to referral to hospitals. The loss in precision expected, because of sampling of controls, was kept small by doubling the number of controls per case. A higher number of controls was not chosen because of practical considerations.

The response rate reached a satisfactory 66.4%. The drop-out analysis did not detect any difference between respondents and non-respondents regarding age, sex, and type of allergy; thus there is little likelihood that these factors influence results. A significantly larger part of the polysensitized group agreed to participate. Further, patients tested in the recent years were more likely to participate than were patients tested in the first years. Patients with severe and/or

present disease may be more likely to participate in studies [160]. The absolute values of clinical characteristics may be biased towards longer and / or more persistent dermatitis, higher frequency of dermatitis / leg ulcers or more generalized dermatitis. If mild cases were missed, and this was more pronounced for the patients with 1-2 contact allergies, any true difference between cases and controls would most likely shift towards the null hypothesis.

5.4.2.2. Confounding

Matching on age and sex was performed to minimize any confounding effect of age and sex. Matching on patch test year was performed to minimize any confounding effect of different exposures, different referral practices and therefore different cohort composition, and differences in patch test materials used related to time. Confirmatively, no difference in age, sex or patch test year distribution was detected between cases and controls in the final material. Other confounding effects, e.g. exposure to irritants and occupational skin disease, were not ascertained and may have differed between cases and controls.

5.4.2.3. Information bias

Attempts to minimize information bias were made when the questionnaire was constructed and validated. One limitation to the validation process was that no test-retest was performed to assess reliability.

Determination of cases and controls was based on number of positive patch test reactions to the last patch test performed in the hospital sector. New allergies may subsequently have developed, causing some controls to be misclassified as such. It is a possibility that development of additional allergies did not end with renewed referral and therefore remained undetected to us. If a large part of the control population was erroneously categorized as such, any true difference between cases and controls would shift toward the null hypothesis.

5.4.2.4. Recall bias

Recall decreases with time [161]. Much recall bias is a general problem which affects all people to some extent. Such recall bias is most likely also present in our study but tends to be non-differential. Relapses may be confused with debuts. Self-reported diagnoses may be underestimated, especially if the disease occurred many years ago or was mild. Outbreak frequency may be both over- and underestimated. Our study subjects were asked to report all skin sites affected with dermatitis at time of debut, but some may have reported all skin sites affected throughout the entire period with dermatitis.

Patients referred to hospital departments are probably more likely to be aware of antecedent exposures or events compared with patients not referred to hospitals [162]. Controls and cases were drawn from the same hospital population to reduce both non-differential and differential recall bias. More patients tested in the recent years responded, which also increased accuracy of information. The accuracy or completeness of information between cases and controls that could stem from differences in age, sex or time span from study to patch test year was reduced by matching. Despite these initiatives, we cannot entirely eliminate recall bias. The extent of recall bias is unpredictable.

5.4.2.5. Definitions

The chosen arbitrary division of the skin surface affects the outcome of the analyses. For the face, none of the subdivisions was associated with polysensitization. In a former study, 23.2% of polysensitized patients had dermatitis on the face [20]. Combining all face-related regions into one variable did, however, not change the results. The trunk was also divided into several regions. Combining the shoulders, chest, stomach, back and buttocks into one region of the trunk showed no association with polysensitization.

For the variable educational level, subjects who could not be categorized, where education was on-going or where data were missing, were grouped into one level. We acknowledge that this level constitutes a highly heterogeneous group. It would have been more correct to categorize subjects with on-going education into one separate group, but only 20 subjects reported on-going education. The variable educational level reflects skills and qualifications obtained from school and vocational education. It does not reflect work-acquired skills acquired by experience and/or informal training. Further, it does not reveal anything about an individual's connection to the labour market. It is acknowledged that the chosen nomenclature carries such inherent limitation. Other classification systems, including national systems, are developed to assess socioeconomic status and are based on occupation [163-165]. They reflect both educational level and work-acquired qualifications and skills and inherently give a more detailed and precise categorization of socioeconomic status. These classification systems were not chosen because we lacked the information required for correct classification.

5.4.2.6. Validity of questions

Self-reported diagnosis was used to determine the prevalence of both dermatitis and other skin diseases (leg ulcers) in the test population. Sensitivity and specificity for self-reported diagnosis has been estimated for hand dermatitis and for skin complaints without specification. Sensitivity of 53-87% and specificity of 79-99% for self-reported diagnosis of hand dermatitis has been reported [166-168]. Sensitivity of 60-80% and specificity of 90-100% has been reported for skin complaints without specification [169-171]. Self-reported diagnoses of different skin diseases tend to underestimate the prevalence but not in one study for dermatitis [172]. Patients self-report of skin complaints and final diagnosis is also correlated with type of skin disease, being high for atopic eczema and seborrhoeic dermatitis and low for skin cancer and acne vulgaris [170]. An alternative way to assess dermatitis and other skin diseases is symptom-based diagnoses. Symptom-based diagnoses tend to overestimate the prevalence with sensitivity rates of 62-100% and specificity rates of 46-87% [166;168;173]. Prior treatments and impairment of social life increase the awareness of diagnosis [172]. It was assumed, but not validated, that self-reported diagnoses of dermatitis may reach higher levels of sensitivity and specificity when used in patch test populations because of increased perception and awareness of skin disease.

Validated questions on clinical characteristics of dermatitis are sparse. The site of skin complaints in general was validated in one study and showed a sensitivity of 67-88% and a specificity of 95-98% for hands, face, arms, legs and body [170]. The reliability ranged from 0.65 to 0.81 when examined 6-8 weeks apart [170]. Questions on school or vocational education have not been validated.

The UK working party criteria were used to assess the occurrence of atopic eczema. The UK working party criteria have been validated in adult [174] and children populations [174;175], and hospital [174] and general populations [175]. The criteria work well in adult outpatients with a sensitivity of 92% and a specificity of 85% [174]. Validation in adult outpatients was performed on consecutive patients including patients with atopic eczema, dermatitis and other dermatoses. AE was therefore assessed in relation to other dermatoses, which could easily be confused with atopic eczema [174]. Most false positives were assessed as inactive cases of AE [174]. Alternative ways to assess atopic eczema were not chosen because the sensitivity and specificity declined. The question “Have you ever suffered from childhood eczema?” had a specificity of 71% and a sensitivity of 90% and overestimated the prevalence in an adult general population [176].

6. CONCLUSION

This PhD thesis contributes to the characterisation of the patient with multiple contact allergies. Patients with multiple contact allergies make up 5% in a patch test population. They have been a stable entity over the last 20 years without any signs of diminishing despite legislative measures. They are primarily elderly women. A large part suffers from atopic eczema and only a minor part from leg ulcers. They did not represent certain educational levels. The majority have hand dermatitis. Dermatitis on other body sites was also linked with polysensitization but not as consistently as was hand dermatitis. The extent of bodily involvement of dermatitis at time of debut was not greater in polysensitized patients. A part of patients with multiple contact allergies will most likely have occupational skin disease, but this was not investigated in the thesis and remains to be elucidated. Most patients with multiple contact allergies were diagnosed with the first patch test in the hospital sector, making primary prevention less relevant in the hospitals. Patients with multiple contact allergies seem approximately equally susceptible to all substances in the European Baseline Series of equal sensitizing capacity and are not directed towards specific chemical configurations. Overall, they have a long duration of disease, respectively 22 and 33 years, dependent on whether they also have atopic eczema (AE), but the contact allergies had a different impact on duration and course of disease dependent on a patient's AE status. An almost complete MOAHLFA index for the polysensitized patient can be generated based on the results presented in this PhD thesis. It is presented in TABLE 19. For the sake of comparison, the MOAHLFA index for patients with 1-2 contact allergies is also presented in the same table.

Leg ulcer patients still carry a great risk of polysensitization even though they may not constitute a large fraction of the entire population of patients with multiple contact allergies. Other factors associated with increased risk of polysensitization were identified and are presented in TABLE 20. The time/cause-relationship could not be further evaluated for the duration of disease or for body sites affected with dermatitis because of the chosen study design. These factors may predispose to polysensitization or may be caused by polysensitization. Patients with multiple contact allergies were more frequently patch tested additional times in comparison with patients

INDEX		1-2 contact allergies	≥ 3 contact allergies
M	Male	27.0%	**22.7%**
O	Occupational	Not examined	**Not examined**
A	Atopy	31.0%	**45.1%**
H	Hand dermatitis	60.7%	**70.1%**
L	Leg *ulcers*	6.7%	**8.6%**
F	Face dermatitis *	33.6%	**34.3%**
A	Age (median, IQR)	47.9 (26.9)	**53.3 (26.0)**

* Demarcation of minimum one of the following regions:
Periorbital region, periauricular region, perioral region, remaining part of face

TABLE 19: *MOAHLFA index for polysensitized patients*

Increased risk of polysensitization
- Female sex
- Increasing age
- Leg ulcers / stasis dermatitis
- Specific allergens - SL mix - Parabens mix - IPPD - Wool alcohols - Cobalt chloride - Potassium dichromate - Myroxylon pereirae
- Repeated patch tests
- Atopic eczema
- Increasing duration of dermatitis
- Hand dermatitis
- Axillary dermatitis
- Back dermatitis

TABLE 20: *Factors associated with increased risk of polysensitization*

with 1-2 contact allergies, which weakly indicates that the multiple allergies cause long duration of disease. Conversely, the risk of additional allergies increased with multiple testing. Some allergens are associated with polysensitization but whether they typically present as primary or secondary allergies is unknown. They seem to be surrogate markers for associated exposure.

Patients with multiple contact allergies are more easily sensitized, show greater elicitation responses and occur more frequently than expected from single sensitivities [1;6]. A few genetic markers are associated with polysensitization [49;50]. These factors still point towards an inherent, increased susceptibility in polysensitized patients. We were not able to detect a unique elicitation response profile in polysensitized patients; however, the study carried some inherent limitations. Evidence of environmental exposure as a key determinant was presented as the risk of multiple contact allergies increased with age and the allergen prevalence patterns in polysensitization primarily seemed to be determined by environmental exposure. The question of whether polysensitization represents a phenotype with inherent, increased susceptibility remains unanswered. Further research into the multifactorial pathogenesis is warranted.

The conclusions of the individual manuscripts are as follows:

I. Patients with multiple contact allergies constituted a minor, but stable, part of a patch test population. Four fifths were women. The risk of polysensitization increased with additional patch testing and with increasing age. Cumulative environmental exposure seemed to be an important causal factor. 90% were already polysensitized at first patch test, making primary prevention less relevant in the hospital sector.

II. No common denominator for the association between the allergens and polysensitization was apparent, except for associated exposure. Any association, whether positive or negative, was

relatively low. The order of development of specific contact allergies is not known. Sensitization to specific baseline allergens cannot be used as risk indicators for polysensitization. The individuals in the contact-allergic population have already displayed an ability to acquire contact allergies and this ability increases the risk of polysensitization rather than the specific allergens themselves.

III. No difference in elicitation dose-response curves, measured as the ratio between ED_{50}, could be detected between polysensitized and single/double-sensitized individuals. Polysensitized patients could therefore not be regarded as more reactive. Some limitations in methodological design and test size were present and the study results should be regarded as exploratory.

IV. Polysensitized patients suffer from dermatitis, nearly every second patient has AE. Long duration of disease was associated with polysensitization but it cannot be determined whether long duration of disease was a cause or consequence of polysensitization. AE was the only identified risk factor for polysensitization. Leg ulcers and educational level did not seem to be risk factors for polysensitization. Patients with AE were overrepresented in the group of polysensitized patients and polysensitized patients should be viewed in the light of occurrence or lack of AE.

V. Hand dermatitis was the only skin site associated with polysensitization regardless of AE status. Special awareness in patients with hand dermatitis is recommended to prevent development of multiple contact allergies or to document polysensitization as an aetiological factor.

7. FUTURE RESEARCH

Future studies on patients with multiple contact allergies may benefit from prospective designs where time-cause relationships can be elucidated. Future studies on patients with multiple contact allergies may also benefit from ordinal designs.

The question of whether polysensitization represents a phenotype with inherent, increased susceptibility still stands, and a replication of the study [1] on induction and elicitation dose-responses in polysensitized individuals is highly desirable. Polysensitization developed because of intense exposure should be avoided or be included as a special subset because of the uncertainty regarding heterogeneity within the polysensitized group. An expansion to include not only allergens but also irritants would be interesting.

With our current knowledge of causal mechanisms for development of contact allergies, genetics play only a minor role at a superior level. However, for polysensitized individuals general exposure regulation in the form of legislative regulations and individual protection measures to minimize exposure to known sensitizations may not be enough. Individual protection has a discouraging compliance [22;177], and allergen avoidance may be unrealistic when patients have several contact allergies and might hold these persons in a chronic state of disease. Elucidating the underlying (multifactorial) genetic pathogenesis of contact allergy will be a valuable contribution for this patient group. An obvious candidate gene is the profilaggrin gene. Mutations in the profilaggrin gene have recently been linked with atopic eczema [146] and ichthyosis vulgaris [178]. Filaggrin mutations lead to lack of the protein filaggrin in the epidermis and impaired skin barrier with increased penetration of environmental substances [179]. It raises the possibility that filaggrin mutations might also predispose to allergic contact sensitization [180]. Identification of other candidate genes can be guided by genome-wide scans.

In manuscript IV, the frequency of dermatitis increased with increasing number of contact allergies, which indicates that single contact allergies are more often accidental findings without clinical relevance compared with multiple contact allergies. As previously mentioned, circumstantial evidence also points towards an increased susceptibility for polysensitized patients [1;6]. Future research on the genetic basis of individual predisposition could benefit from using polysensitized individuals as point of reference. The likelihood of identifying genetic markers seems the greatest in this cohort. Polysensitized patients with high skin exposure should be excluded as this may reduce the impact of genetic polymorphisms or mutations on development of contact allergies and shift the results toward null hypothesis. The only limitation to using polysensitized patients as representatives for the entire contact allergy cohort is the rare occurrence of polysensitization. Large cohorts are needed for detection of genetic effects. In a cohort of 14,998 patients collected over 20 years, only 759 patients had multiple contact allergies. This problem can be overcome by a multicentre-design.

8. SUMMARY

8.1. Summary in English

This PhD thesis deals with patients with multiple contact allergies. Multiple contact allergies are defined as contact allergy to 3 or more chemical substances and are synonymous with polysensitization. The superior aim of the thesis was to contribute to a better characterization of the group of patients with multiple contact allergies, and within this to 1) examine the prevalence (manuscript I, study part I); 2) describe the demographic characteristics (manuscript I, study part I); 3) describe subgroups (manuscript IV, study part III); 4) examine the type of allergies and associations between allergens and polysensitization (manuscript II, study part I); 5) examine clinical characteristics such as site of dermatitis (manuscript V, study part III), course and duration of disease (manuscript IV, study part III); and 6) examine the elicitation response at challenge with specific allergens in patients with multiple contact allergies (manuscript III, study part II).

The thesis consists of 3 study parts. Study part I is an epidemiological study based on data from 14,998 individuals patch tested with the European Baseline Series at one hospital department during 1985-2005. Study part II is an experimental dose-response study, which included 13 polysensitized individuals and 38 individuals with 1-2 contact allergies. Study part III is a questionnaire study conducted on 394 polysensitized individuals and 726 matched controls with 1-2 contact allergies, all from the same cohort as used in study part I.

The studies showed that the frequency of polysensitization over 20 years was stable. The prevalence was 5.1%. Four out of 5 with multiple contact allergies were women and the risk of polysensitization increased with increasing age; 90% were diagnosed with multiple contact allergies at the first patch test in the hospital sector. When repeating the patch test, the risk of multiple contact allergies increased. Around every 9th patient with multiple contact allergies was patch tested several times, which indicates persistent or recurrent dermatitis. Those allergens that are known to be frequent causes of contact allergy were also frequent causes among patients with multiple contact allergies and vice versa for the rare allergens. Several allergens were, respectively, positively and negatively associated with polysensitization, but no clear pattern of associations was found.

In patients with multiple contact allergies, 45% had had atopic eczema, whereas the occurrence of leg ulcers was low. The hands were the body part which was affected with dermatitis most frequently at time of debut. Patients with multiple contact allergies did not have more widespread dermatitis than did patients with 1-2 contact allergies. Atopic eczema and hand dermatitis were positively associated with polysensitization. Neither specific educational levels nor leg ulcers increased the risk of polysensitization. Other body parts were also, respectively, positively and negatively associated with polysensitization, but this was not as consistent as with hand dermatitis. The number of contact allergies had a different influence on duration and course of disease among patients with and without atopic eczema, respectively.

At elicitation with allergens in dilution series, patients with multiple allergies did not react at lower concentrations than did patients with 1-2 contact allergies. Therefore, patients with multiple contact allergies could not be viewed as more reactive.

The studies have contributed with demographic and clinical data about patients with multiple contact allergies and new risk factors have been identified. Future research in patients with multiple contact allergies can profit from using prospective designs, and, for example, genetic association studies can profit from using patients with multiple contact allergies as point of reference.

8.2. Summary in Danish

Denne pHD-afhandling omhandler patienter med multiple kontaktallergier. Multiple kontaktallergier er defineret som kontaktallergi over for 3 eller flere kemiske stoffer og er synonymt med polysensibilisering. Afhandlingens overordnede formål var at bidrage til en bedre karakteristisk af gruppen af patienter med multiple kontaktallergier herunder 1) at undersøge prævalensen (manuskript I, delstudium I); 2) beskrive demografiske karakteristika (manuskript I, delstudium I); 3) beskrive subgrupper (manuskript IV, delstudium III); 4) undersøge typen af allergier og associationer mellem allergener og polysensibilisering (manuskript II, delstudium I); 5) undersøge kliniske karakteristika som eksemlokalisation (manuskript V, delstudium III); sygdomsforløb og varighed (manuskript IV, delstudium III); og 6) undersøge det allergiske respons ved elicitering med specifikke allergener hos patienter med multiple kontaktallergier (manuskript III, delstudium II).

Afhandlingen består af 3 delstudier. Delstudium I er en epidemiologisk undersøgelse baseret på data fra 14.998 personer lappetestet med den europæiske baseline serie ved én hospitalsafdeling i perioden 1985-2005. Delstudium II er et eksperimentelt dosis-respons studium, der inkluderede 13 polysensibiliserede individer og 38 individer med 1-2 kontaktallergier. Delstudium III er en spørgeskemaundersøgelse udført på 394 polysensibiliserede individer og 726 matchede kontroller med 1-2 kontaktallergier, alle fra samme kohorte anvendt til delstudium I.

Studierne viste, at hyppigheden af polysensibilisering gennem de sidste 20 år var stabil. Prævalensen var 5.1%. Fire ud af 5 med multiple kontaktallergier var kvinder, og risikoen for polysensibilisering steg med stigende alder; 90% fik konstateret multiple kontaktallergier ved første lappetest i hospitalssektoren. Ved gentagen lappetest øgedes risikoen for multiple kontaktallergier. Ca. hver 9. patient med multiple kontaktallergier blev lappetestet flere gange som udtryk for vedvarende eller tilbagevendende eksem. De allergener, der er kendt som hyppige årsager til kontaktallergi, var også hyppige årsager blandt patienter med multiple kontaktallergier og vice versa med sjældne allergener. Flere allergener var henholdsvist positivt og negativt associeret med polysensibilisering, men der kunne ikke findes noget entydigt mønster for disse associationer.

45% af patienter med multiple kontaktallergier har haft atopisk eksem, hvorimod forekomsten af bensår var lav. Hænderne var den kropsdel, der oftest var afficeret med eksem ved debut. Patienter med multiple kontaktallergier havde ikke mere udbredt eksem end patienter med kun 1-2 allergier. Atopisk eksem og håndeksem var positivt associeret med polysensibilisering. Hverken

specifikke uddannelsesniveauer eller bensår øgede risikoen for polysensibilisering. Andre kropsdele var også henholdsvist positivt og negativt associeret med polysensibilisering men dog mindre konsekvent end ved håndeksem. Antallet af kontaktallergier havde forskellig effekt på varighed og forløb af eksemsygdom blandt patienter henholdsvis med og uden atopisk eksem.

Ved provokation med allergener i fortyndingsrække reagerede patienter med multiple allergier ikke ved lavere koncentrationer end patienter med 1-2 kontaktallergier. Patienter med multiple kontaktallergier kunne derfor ikke betragtes som mere følsomme.

Undersøgelserne har bidraget med demografiske og kliniske data om patienter med multiple kontaktallergier og nye risikofaktorer er blevet identificeret. Fremtidig forskning i patienter med multiple kontaktallergier kan drage fordel af at anvende prospektive designs og for eksempel genetiske associationsstudier kan med fordel tage udgangspunkt i patienter med multiple kontaktallergier.

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10. MANUSCRIPTS I - V

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CONTACT DERMATITIS

20 Years of standard patch testing in an eczema population with focus on patients with multiple contact allergies

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Results of standard patch tests performed with the same methodology in one centre are rarely available over a large time span. This gives the unique opportunity to study not only prevalence but also persistency of contact allergy and characterize subpopulations. The objectives were to investigate sensitivity rates and persistencies of patch test results and characterize patients with multiple contact allergies. A 20-year retrospective database-based study of 14 998 patients patch tested with the European Standard Series was performed. 34.5% were sensitized, primarily women. Sensitivity to nickel was most frequent and least frequent to mercaptobenzothiazole, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine and benzocaine. Yearly proportion of negative, mono/double-allergic, and multiple-allergic cases remained stable. Persistency of positive reactions was high for para-phenylenediamine, Cl(Me)isothiazolinone, and primin and poor for paraben mix. 5.1% were multiple allergic, primarily women, and 90% got diagnosed by the first test. Frequency of multiple allergies increased with age. More multiple- than mono/double-allergic patients were tested multiple times. Persistency and sensitivity rates in a Danish eczema population are provided and are useful for decisions regarding the standard series. Patients with multiple contact allergies are typically elderly women who might have long-lasting and hard-to-treat eczema. Cumulative environmental exposure seems necessary to develop multiple allergies.

Key words: contact allergy; database; European Standard Series; multiple contact allergies; multiple patch tests; persistence; persistency rate; polysensitization; sensitivity rate; sensitization.

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Contact allergy is a delayed-type hypersensitivity reaction triggered by direct skin contact with low-molecular weight molecules in the environment. Contact allergy is generally perceived as being life-long, and patients are advised to avoid specific allergens for the rest of their lives. In contrast, studies on persistency of patch test results with varying time lapse between tests show a more complex picture with persistency rates less than 100% (1–3).

The European Standard Series is composed of 23 allergens. The European Standard Series detects approximately 75% of all contact allergies as about 15% of individuals tested are positive to other allergens only and 10% are positive to both allergens in the Standard Series and other allergens (4). Variations in sensitivity rates exist within Europe (5) and even between different patch-testing centres in the same country (6). In Denmark, legislation of nickel release from consumer objects and addition of ferrous sulfate to cement have had marked impact on the prevalence of nickel and chrome sensitivity (7). Sensitivity rates for allergens in the European Standard Series have not previously been reported for our region and patient mix.

Patients with multiple contact allergies have received limited research focus. Knowledge about patients with multiple contact allergies mainly relies on a few studies and general perceptions. Patients with multiple contact allergies probably do exhibit a special entity in the field of contact allergies as the observed frequency exceeds the predicted frequency in both general (8) and eczema populations (9).

All results from individuals patch tested since 1979 have been electronically registered in a database at the Copenhagen University Hospital Gentofte, Denmark. Using the information gathered in the database, the aim of this study is to

investigate sensitivity rates and persistency of positive patch test results over time and to identify and characterize patients with multiple contact allergies within a Danish eczema population.

Methods

At the Department of Dermatology, Copenhagen University Hospital Gentofte, Denmark, patients suspected of suffering from allergic contact dermatitis are tested with the European Standard Series and, if relevant, additional allergens dependent on exposure. All the patch test results are consecutively registered in a database. Patch test results from 1985 to 2005 regarding 23 allergens contained in the European Standard Screening Tray were selected and analysed.

In the entire period, patch testings were done using Finn Chambers® and Scanpor tape applied to the upper back. The occlusion time was 48 hr. Readings were done on D2, D3 or D4, and D7 according to the recommendation from the International Contact Dermatitis Research Group (10). Homogeneous redness and infiltration in the entire test area was scored as a 1+ reaction. Homogeneous redness, infiltration, and vesicles in the test area were scored as a 2+ reaction, and homogeneous redness, infiltration, and coalescing vesicles in the test area as a 3+ reaction.

A 1+, 2+, or 3+ reading was interpreted as a positive response. An irritative response, doubtful (+?), or negative reading was interpreted as a negative response. Not all patients were tested with the complete standard series because of known sensitivity diagnosed by previous patch test at the Department. A registration of known sensitivity was interpreted as a positive response. A few patients were not tested to the complete standard series of unknown reasons. This registration was categorized as missing data.

Patients and Materials

14 998 patients (63.6% women and 36.4% men) were patch tested. Median age at first test was 47.40 years [interquartile range (IQR) 28.87]. Stratified into 5 age groups 1.7% (255) patients were under 16 years of age at first patch test, 20.3% (3041) were between 16 and 30 years, 34.8% (5212) were between 31 and 50 years, 29.6% (4437) were between 51 and 70 years, and 13.7% were over 70 years of age at the time of first patch test.

86.2% (12 926) of the patients were patch tested with all 23 allergens or had a known positive reaction diagnosed by a previous patch test performed at the Department. The lowest number of allergens tested per individual was 16 which only concerned 3 patients. The sesquiterpene lactone cocktail was unavailable for testing during 1985–86. Excluding the missing data caused by this circumstance, 95.5% of all patients were tested with all standard allergens available on the time of testing or had a known positive reaction diagnosed by a previous patch test.

16 allergens did not change concentration or composition during the 20-year period. In, respectively, 1988, 1986, and 1995, para-phenylenediamine (PPD) changed concentration from 0.5% to 1%, colophony changed concentration from 60% to 20%, and mercapto mix changed concentration from 2% to 1%. 2 allergens changed composition. Quinoline mix 6% was in 1994 substituted with clioquinol 5%, and black rubber mix 0.6% was in 1993 substituted with *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine (IPPD) 0.1% petrolatum. In 1995 paraben mix changed both composition and concentration as 1 paraben ester was removed and concentration of the remaining 4 esters was changed to 4% each (11). We first started to test with sesquiterpene lactone cocktail 0.1% in 1987. Different allergen concentrations and compositions used were not evaluated separately.

Statistics

The data analysis was done using the statistical software programme SPSS version 13.

6.5% of the patients were tested several times. Overall and allergen-specific sensitivity rates were calculated by using the last patch test performed per individual as point of origin, if not stated otherwise.

Comparison of sensitivity rates was made using the χ^2 test and evaluation of trends with χ^2 for trend. Comparison of age distributions was done using the Mann–Whitney test. Examination of sensitivity rate changes between tests on the same population was done using the McNemar nonparametric test based on binomial distribution. Because of multiple testing, when comparing sex-specific sensitivity rates for each allergen, the P value was adjusted according to the method of Bonferroni so that a P value below 0.002 was regarded as significant. For the remaining calculations a P value below 0.05 was regarded as significant.

Only those patients who had been tested consecutively with the specific allergen of interest were evaluated for the analysis of persistency of positive patch test reactions.

The influence of sex and age on the occurrence of multiple contact allergies was evaluated by

nominal, ordinal, and binary logistic regression models. The occurrence of multiple contact allergies was used as dependent outcome variable, and age, sex, and interaction between age and sex, were used as independent variables. To test if the binary model fitted the data adequately, Hosmers goodness-of-fit test was used and the fit was adequate. The nominal and ordinal regression model was performed because the binary model simplified the data. The nominal and ordinal regression model resulted in almost identical results. The nominal regression model was run with maximum likelihood estimation, and the ordinal model was run with weighted least-square estimation. The almost identical results of the nominal and ordinal regression model indicated that the conditions for the ordinal model had been fulfilled.

Results

Trends

The total number of patients patch tested each year increased steadily (Fig. 1); however, the proportion of positive and negative cases per year remained stable (Fig. 2). Distributing the positive cases into patients with 1–2 contact allergies and patients with ≥ 3 contact allergies, the proportion of both groups also remained stable over the years as illustrated in Fig. 2 with parallel trend lines (χ^2 for trend, $P = 0.647$). Age median increased by approximately 5 years from 1985 to 2005 (results not shown). The fraction of women tested each year varied greatly; overall, showing only a marginal increase from 1985 to 2005 (results not shown).

Allergies in an eczema population

In total, 34.5% (5178) had at least 1 contact allergy (range 1–12). 40% of the women tested had a contact allergy versus 25% of the men tested (χ^2, $P < 0.001$). As more women than men were tested, 73.7% of all diagnosed allergies were among women.

Positive reactions to all the 23 allergens or mixes of allergens in the standard series were found. The sensitivity rate for each specific allergen is illustrated in Table 1. Sensitivity was most frequent to nickel (12.0%) and least frequent to mercaptobenzothiazole (MBT), IPPD, and benzocaine (for all three 0.5%).

Significantly more women than men were sensitized to cobalt, formaldehyde, colophony, *p*-tert-butylphenol-formaldehyde resin (PTBFR), fragrance mix, quaternium 15, nickel, Cl(Me)isothiazolinone (MCI/MI), primin, and sesquiterpene lactone cocktail (Table 1) (Bonferroni-adjusted χ^2, $P < 0.002$).

Persistency of positive patch test reactions

Persistency of positive patch test reactions means that a positive reaction observed at one patch test is reproduced at a second patch test performed at a varying time span after the first test. The degree of persistency of positive patch test reactions differed between the allergens. In Table 2 allergens are ranked in 5 categories according to persistency: poor (<20%), fair (21%–40%), moderate (41%–60%), good (61%–80%), and very good ($\geq$81%). 9 of 23 allergens ranked as good or very good, whereas only 5 allergens ranked as poor or fair. PPD, MCI/MI, and primin had a very high degree of persistency ($\geq$81%). In contrast paraben mix had a poor persistency (11%).

Multiple contact allergies

Multiple contact allergies were defined as 3 or more than 3 contact allergies. 759 patients

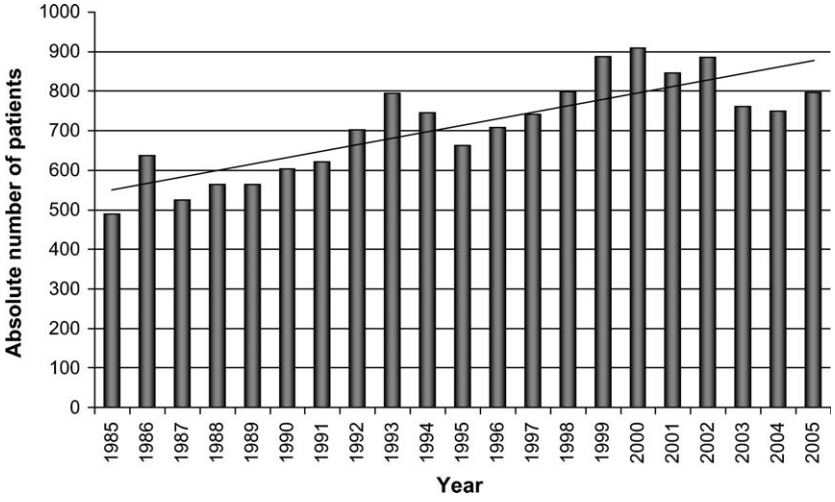

Fig. 1. Total number of patients patch tested with the European Standard Series between 1985 and 2005 at the Department of Dermatology, Copenhagen University Hospital Gentofte, Denmark. Trend line interposed.

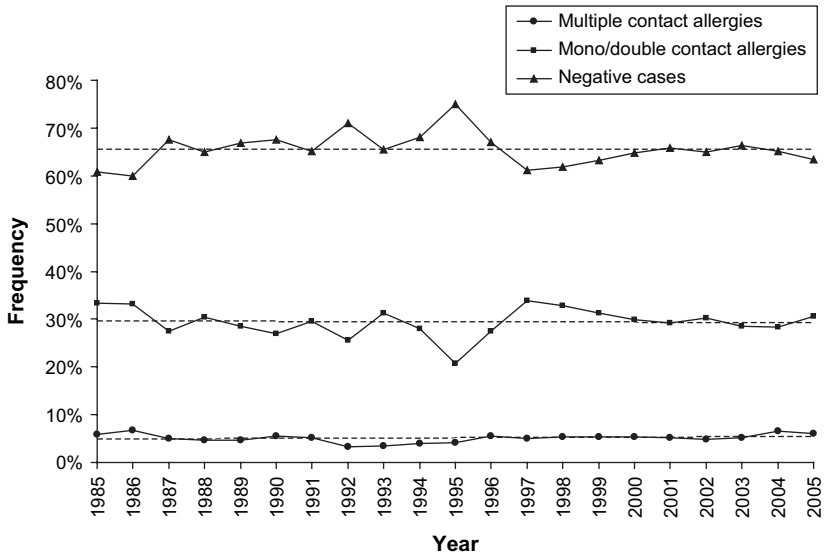

Fig. 2. Proportion of negative, mono/double-allergic, and multiple-allergic patients each year from 1985 to 2005 tested with the European Standard Series. Trend lines are illustrated for each reaction group.

(77.3% women and 22.7% men) had multiple contact allergies, correlating to a prevalence of 5.1%. 683 individuals had multiple contact allergies at time of first patch test, correlating to 90% of all diagnosed multiple-allergic individuals. Median age at time of diagnosis of multiple contact allergies was 52.98 years (IQR 26.50). The rate of patients with multiple contact allergies increased with age (Table 3). The frequency of 1–2 allergies did not show the same increasing trend with age (Table 3).

The occurrence of multiple contact allergies was significantly associated with sex and age and sex × age in combination (nominal regression model, for all 3 factors $P < 0.0001$). The sensitivity rates of multiple contact allergies at different ages and sex are illustrated in Fig. 3.

Table 1. Total and sex-specific sensitivity rates for each allergen in the European Standard Series 1985–2005

Allergens (*n*)	Men (%)	Women (%)	Total (%)
Chrome (14 979)	2.3	2.5	2.4
Neomycine (14 978)	2.6	2.9	2.8
Thiuram mix (14 982)	2.3	2.9	2.7
Para-phenylenediamine (14 966)	1.8	2.3	2.1
Cobalt (14 976)	2.4	4.3	3.6*
Benzocaine (14 995)	0.3	0.7	0.5
Formaldehyde (14 980)	2.2	3.2	2.9*
Colophony (14 985)	3.1	4.4	3.9*
Clioquinol (14 996)	1.0	0.6	0.7
Balsam of Peru (14 988)	3.7	4.8	4.4
N-isopropyl-*N*-phenyl-*p*-phenylenediamine (14 964)	0.7	0.4	0.5
Wool alcohols (14 994)	1.0	0.8	0.9
Mercapto mix (14 989)	0.8	0.6	0.7
Epoxy resin (14 987)	1.3	1.0	1.1
Paraben mix (14 996)	0.7	0.5	0.6
p-tert-butylphenol-formaldehyde resin (14 994)	0.8	1.4	1.2*
Fragrance mix (14 971)	5.9	8.8	7.7*
Quaternium 15 (14 993)	0.5	1.1	0.9*
Nickel (14 845)	3.1	17.2	12.0*
(Cl)Me-isothiazolinone (14 878)	1.2	2.1	1.8*
Mercaptobenzothiazole (14 852)	0.6	0.5	0.5
Primin (14 986)	0.3	1.6	1.1*
Sesquiterpene lactone cocktail (13 198)	0.8	1.5	1.2*

Significant difference in sensitivity rate between sex exists for 10 allergens marked with asterisk (χ^2 test, $P < 0.002$). *n*, total number of patients tested.

Multiple testing

Mainly patients with chronic and recurrent dermatitis may be subjected to retests. 977 individuals (67.6% women and 32.4% men) were patch tested between 2 and 5 times. 153 (15.7%) had 3 or more than 3 contact allergies and 347 (35.5%) had 1 or 2 allergies. 477 (48.8%) did not have any contact allergies.

The median age, fraction of women, and proportion of multiple-allergic patients increased with number of tests performed (Table 4). There were significantly more women and more mono/double- and multiple-allergic individuals and a higher age median at time of first test in the multiple-tested group compared with the single-tested group (Table 4). 11.2% of patients diagnosed with multiple contact allergies and 7.6% of patients diagnosed with mono/double contact allergies at time of first test ended up being tested multiple times (χ^2, $P < 0.001$).

Discussion

We report results from a Danish contact allergy database. It is unique as it compiles results from 20 years of patch testing in a specialist unit. We found a 34.5% sensitivity rate of contact allergy in

Table 2. Persistency rates, positives in first and second patch test and new and lost positives in the second patch test for each allergen in the European Standard Series

Rank	Allergens (n)	Persisted positive (%)	Positives in first test	Positives in second test	Lost positives	New positives
Very good	Primin (964)	10 (91)	11	11	1	1
	MCI/MI (956)	7 (88)	8	23	1	16
	PPD (962)	5 (83)	6	30	1	25
Good	Neomycine (959)	17 (68)	25	27	8	10
	Quaternium 15 (977)	6 (67)	9	13	3	7
	Benzocaine (976)	4 (67)	6	10	2	6
	IPPD (971)	4 (67)	6	12	2	8
	Mercapto mix (966)	2 (67)	3	9	1	7
	SL mix (817)	8 (62)	13	23	5	15
Moderate	Cobalt (954)	13 (57)	23	38	10	25
	Epoxy resin (967)	4 (57)	7	9	3	5
	Fragrance mix (919)	31 (57)	54	87	23	56
	Colophony (934)	20 (56)	36	50	16	30
	Nickel (887)	26 (54)	48	61	22	35
	Chrome (957)	10 (48)	21	36	11	26
	Balsam of Peru (944)	11 (48)	23	56	12	45
	Formaldehyde (956)	9 (43)	21	30	12	21
	Thiuram mix (954)	10 (42)	24	45	14	35
Fair	PTBFR (969)	3 (38)	8	7	5	4
	MBT (957)	1 (33)	3	5	2	4
	Wool alcohols (968)	5 (31)	16	16	11	11
	Clioquinol (969)	2 (29)	7	11	5	9
Poor	Paraben mix (976)	1 (11)	9	8	8	7
	Total	209 (54)	387	617	178	408

Median time lapse between first and second test was 4.4 years for SL mix, 4.7 years for cobalt and formaldehyde, and 4.8 years for the rest of the standard allergens. n, total number of patients tested. IPPD, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine; MBT, mercaptobenzothiazole; MCI/MI, Cl(Me)isothiazolinone; PPD, para-phenylenediamine; PTBFR, *p*-tert-butylphenol-formaldehyde resin; SL mix, sesquiterpene lactone cocktail.

Table 3. Age and sex distribution of patients with 0, 1–2 and ≥3 contact allergies at the time of first test

Age	No allergies (%) ($n = 9909$; 42% males, 58% females)	1–2 Allergies (%) ($n = 4406$; 27% males, 73% females)	≥3 Allergies (%) ($n = 683$; 21% males, 79% females)
<16 years	82.0	16.9	1.2
16–30 years	70.3	27.0	2.7
31–50 years	63.3	31.9	4.8
51–70 years	65.2	29.7	5.1
>70 years	66.6	27.3	6.1

Frequency of multiple contact allergies and age are significantly associated ($P < 0.001$). *n*, absolute number of patients.

our eczema population, a rate of multiple contact allergies at 5%, and allergen-specific prevalence correlating well with data from other European centres (5, 9, 12). Nickel is the most frequent allergy followed by fragrance mix and IPPD, benzocaine, and MBT, the rarest allergies. Primarily women get a diagnosis of contact allergy. An inherent female susceptibility has been speculated (13), although female-related behaviour with increased exposure and female-dominated professions with exposure to wet work and other irritants could be explanatory in themselves (14).

Despite an increase in patients tested during the last 20 years, the frequency of positive and negative and mono/double- and multiple-allergic cases each year is stable. From the small increase in age median and marginal increase in fraction of women tested each year over the 20-year period, a slight increase in sensitivity rate could be expected. As this is not the case, a small effective decrease of contact allergy during the 20 years cannot be excluded. 1 out of 6 positive cases had multiple allergies. Only 0.7% of all tested and therefore 1 out of 21 positive cases had multiple contact allergies in a general Danish population (8). The upconcentration of patients with multiple allergies in the eczema population might be related to severe eczema and a need for medical assistance.

From a diagnostic point of view, it is generally appreciated that sensitivity rates of allergens attained in the European Standard Series should exceed 1%. This does not apply to 8 allergens. In a meta-analysis based on 14 studies with TRUE test-based prevalence of allergens (15), 6 of the 8 allergens identified in our study also rank low in frequency (mercapto mix, MBT, quinoline mix, black rubber mix, quaternium 15, and paraben mix). Introduction of new allergens and changes in exposure continuously challenge the justification of allergens chosen to be part of the European Standard Series. As the number of allergens attained in a Standard Series needs to be limited

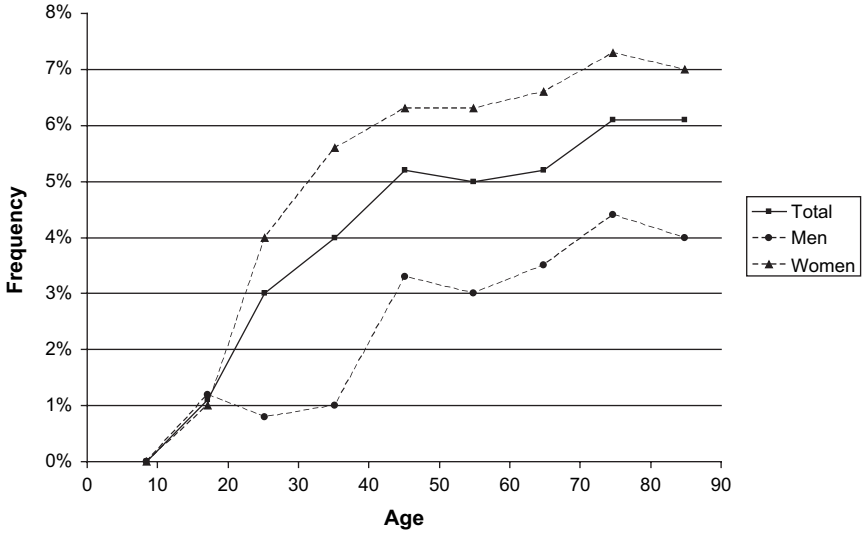

Fig. 3. Association between age and frequency of multiple contact allergies in a Danish eczema patient population at time of first patch test. Sex-specific association is also illustrated. Multiple contact allergies is defined as ≥3 contact allergies.

for practical reasons, only allergens that account for the greatest majority of hypersensitivity reactions should be included.

Overall persistency of positive patch test reactions was 54%. The level of persistency varies in different studies (1–3, 16). Primin, MCI/MI, and PPD had a very good persistency of over 80%. These 3 allergens also have a strong allergenic potency (17). In contrast, some of the allergens with a fair or poor persistency, wool alcohols, clioquinol, paraben mix, have a weak allergenic potency (18–20). The same pattern is demonstrated in one other study (1). Weak sensitizers often give weak-positive reactions which more often turn negative on a second testing (1, 21). But even strong sensitizers such as Dinitrochlorobenzene (DNCB) do not exhibit a persistency of 100% as some positive reactions are lost over time (21). Contact allergy is generally perceived as being lifelong, but the level of persistency of positive patch test reactions is never 100% as our results also confirm.

Contact allergy is a dynamic process demonstrated by fluctuations in the patch test reactivity. The intra-individual variation in patch test reactivity is great, sometimes even resulting in negative test results with positive test results to the same allergen at earlier and later testing (22). Skin responsiveness, skin absorption, and seasonal variations also contribute to fluctuations in patch test results (23–25). They are all important factors for persistency of positive reactions in combination with intra-individual variation and allergenic potency for which we cannot control in everyday dermatology practice. In contrast, the test method, preparation, application of test chambers, defined reading times, and standardized interpretations, all factors susceptible to control, were during the last 20 years unchanged and performed by a very small number of specially trained personnel. This procedure minimizes the risk of inconsistencies in methodology and in interpretation. In addition, routine D7 readings

Table 4. Characteristics of patients patch tested multiple times

Sequential count (*n*)	Age (median age, IQR)	Women (%)	1–2 contact allergies (%)	≥3 contact allergies (%)
First patch test (977)	48.5, IQR 27.4*	67.6†	334 (34.2)‡,§	77 (7.9)¶,**
Second patch test (977)	54.4, IQR 26.2	67.6	359 (36.7)‡	135 (13.8)
Third patch test (116)	56.6, IQR 29.3	78.4	47 (40.5)	28 (24.1)

The fourth and fifth patch test have been omitted due to the low number of patients tested 4 ($n = 15$) and 5 times ($n = 2$). IQR, interquartile range; *n*, number of patients tested.

*Significant difference in age between single-tested and multiple-tested individuals at the time of first test (Mann–Whitney test, $P = 0.04$).

†Significantly more women in the multiple-tested group versus the single-tested group (χ^2, $P < 0.01$).

‡Significant difference in sensitivity rate regarding mono/double contact allergies between first and second test (McNemar, $P < 0.001$).

§Significantly higher sensitivity rate regarding mono/double contact allergies in the multiple-tested group at the time of first test versus the single-tested group (χ^2, $P < 0.001$).

¶Significant difference in sensitivity rate regarding multiple contact allergies between first and second test (McNemar, $P < 0.001$).

**Significantly higher sensitivity rate regarding multiple contact allergies in the multiple-tested group at time of first test versus the single-tested group (χ^2, $P < 0.001$).

were performed for all allergens, supplementing D2 and D3 readings and optimizing the persistency of positive reactions to, e.g. neomycine known to give reactions on D7.

The last official revision of the European Standard Series dates back to 2000 (26). Our observation of low persistency and low sensitivity rates for 4 allergens (paraben mix, clioquinol, wool alcohols, and MBT) do not fulfil the demands on a sensitizer proposed by the European Society of Contact Dermatitis (27). Allergens such as wool alcohols, paraben mix, and clioquinol might be candidates for exclusion. Furthermore, they typically occur among specific subpopulations for which additional testing is often performed (20, 28, 29). In contrast, allergens with sensitivity rates above 1% but with a poor persistency (e.g. PTBFR) are candidates for further research into optimizing the patch test method and material.

Subpopulations of eczema patients with different compositions can be identified in the database. Patients tested several times are more often women, and a larger part of the patients have a contact allergy as the total sensitivity rate is higher at the time of first test in comparison with patients only tested once. The group seems to have an increased susceptibility towards developing additional contact allergies as the total sensitivity rate and, in particular, the rate of patients with multiple contact allergies increase with tests performed. The increased susceptibility could be caused by an inherent ability to develop contact allergy as speculated for patients with multiple contact allergies (9, 23). But persistent skin disease and a defect skin barrier, which multiple-tested patients often have, can also increase susceptibility towards developing additional allergies (24). At the time of first test, there are no known characteristics which can identify patients who end up in this subpopulation. But patients for whom a second testing is needed have an increased risk of having multiple contact allergies, and preventive strategies such as avoiding known allergens as well as minimizing exposure to other frequent contact allergens could be introduced at this time.

It is generally perceived that patients with many contact allergies have a longer duration of disease and perhaps more severe and more widespread eczema. This perception has not to our knowledge been the focus of attention in any study. We show that a larger part of the patients with multiple contact allergies diagnosed at first patch test than patients with mono/double allergies end up being tested multiple times. Patients tested several times typically have more long-lasting and hard-to-treat eczema. The finding indicates that the perception about disease severity and disease duration of multiple-allergic patients is correct. However, the significant difference between the 2 groups is small, and the finding needs to be further investigated in other studies.

Patients with multiple contact allergies are typically elderly women. Patients with 1 or 2 contact allergies exhibit a fairly constant sensitivity rate at different ages, whereas the rate of patients with multiple contact allergies increases with age. If genetics were the determining factor of development of multiple contact allergies, a larger part of the subgroup would probably be younger. A cumulative environmental exposure seems necessary in order to develop multiple contact allergies.

Unfortunately, 90% of multiple-allergic patients get diagnosed at first patch test in the hospital sector excluding the opportunity of primary prevention at this stage. Primary prevention must be executed at an earlier stage in the health care system but characteristics have yet to be established to identify patients at risk of developing multiple contact allergies. It is unknown if the rate is the same or lower when first patch tested in a private dermatology practice.

The population is constituted of eczema patients suspected of having a contact allergy referred to, diagnosed, and treated at a university hospital. The results do not give any information on patients treated in a private dermatology practice, at a primary health facility or on the patterns of contact allergy in the general population. The database provides a unique surveillance opportunity for identifying new culprit allergens or for local quality control of material preparations when fluctuations in sensitivity rates suddenly appear. Information on culprit allergens can lead to preventive strategies as we have seen for nickel (30), and consequences of legislation can be measured. Decreased sensitivity rates can show allergens no longer justified to be in screening series. Different subpopulations can be identified as presented in this study. Clinical epidemiological studies can bring about new information in our understanding of contact allergies. In Denmark we have the unique possibility of linking databases due to a unique personal identifier number assigned to each individual at birth, making identification of individuals over time possible. Results from linking of databases can extend our knowledge of contact allergies as an entity, e.g. in relation to other inflammatory diseases (31).

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CONTACT DERMATITIS

Associations between baseline allergens and polysensitization

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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 pereirae*, 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 methyldibromo glutaronitrile 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, Hosmers 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

	Single/double contact allergies ($n = 4419$)		Multiple contact allergies ($n = 759$)	
Allergens	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
Myroxylon pereirae	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

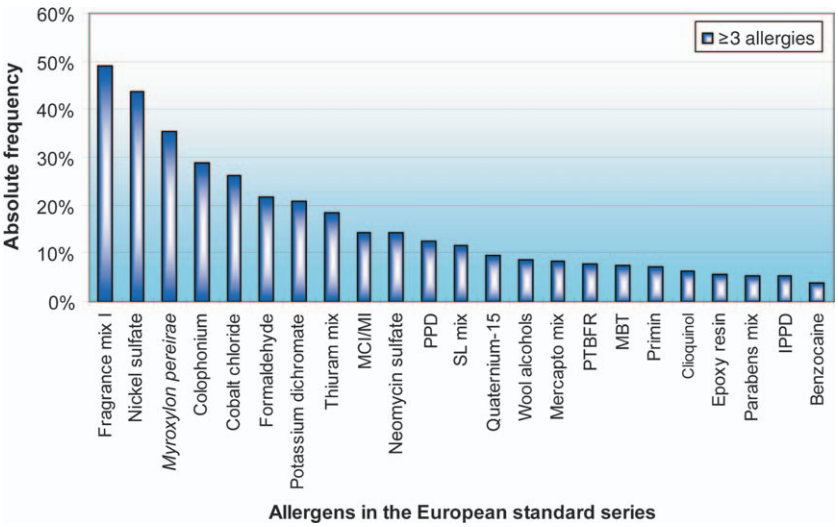

Fig. 1. Allergen sensitivity rates among patients with multiple contact allergies ($n = 759$). IPPD, *N*-isopropyl-*N*-phenyl-*p*-phenylenediamine; MBT, mercaptobenzothiazole; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; PPD, *p*-phenylenediamine; PTBFR, *p*-tertiary butylphenol formaldehyde 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)
Myroxylon pereirae (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.
[a]Mercaptobenzothiazole and quaternium-15 was excluded from count of additional allergies.

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
Myroxylon pereirae	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.
[a]The analyses were adjusted for sex, age, and interaction between sex and age.

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 number 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	*n*	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
*Myroxylon pereirae**	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.
[a]The 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 it 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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Patch test dose–response study: polysensitized individuals do not express lower elicitation thresholds than single/double-sensitized individuals

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Summary

Background It is not known if reduced elicitation thresholds are evident among polysensitized individuals when using allergens to which the patients are already sensitized. Reduced elicitation thresholds may be an expression of increased reactivity in this patient group.

Objectives To examine and compare elicitation dose–response curves and elicitation thresholds in a polysensitized vs. a single/double-sensitized group for allergens to which the test subjects were already sensitized.

Patients/methods Fifty-one patients (13 polysensitized and 38 single/double-sensitized) were patch tested with nickel sulphate, methyldibromo glutaronitrile (MDBGN) and p-phenylenediamine (PPD) in dilution series. The ratio between the doses eliciting a response in 50% of patients in the two groups was used as the measure for relative sensitivity.

Results The dose–response curves of the polysensitized group for MDBGN and PPD were shifted to the right, and for nickel sulphate shifted to the left, compared with the single/double-sensitized group. The relative sensitivity for each of the three allergens and a combined relative sensitivity for all three allergens were not significantly different when comparing the polysensitized and single/double-sensitized groups.

Conclusion No increased sensitivity, in the form of distinct elicitation thresholds, could be demonstrated in polysensitized individuals compared with individuals with one or two contact allergies.

Polysensitized patients appear more frequently than would be expected by chance,[1,2] are more easily sensitized and show greater elicitation responses when experimentally exposed to dinitrochlorobenzene (DNCB).[2] These findings suggest that polysensitized individuals are particularly sensitive to sensitization and immunologically reactive.

It is not known if reduced elicitation thresholds are evident among polysensitized individuals when using allergens to which the patients are already sensitized. Reduced elicitation thresholds may be an expression of increased reactivity in this patient group which may result in severe and long-lasting disease.

The aim of this study was to examine and compare the elicitation threshold and elicitation dose–response curves in polysensitized patients vs. a reference group of single/double-sensitized patients for the allergens nickel sulphate, methyldibromo glutaronitrile (MDBGN) and p-phenylenediamine (PPD).

Materials and methods

Data were compiled from three previous investigations and reanalysed with the specific purpose as described. The three original studies were all approved by the local ethics committee (Copenhagen County) and all study subjects gave their written informed consent before enrolment.

Twenty nickel-allergic test subjects were patch tested with a nickel sulphate dilution series of 19 different concentrations ranging from 0·0000228% to 3% and one ethanol/water control. Eighteen MDBGN-allergic test subjects were patch tested with a MDBGN dilution series of 19 different concentrations ranging from 0·0000077% to 0·5% and one ethanol/water control. Fifteen PPD-allergic test subjects were patch tested with a PPD dilution series of eight different concentrations ranging from 0·0001% to 1% and one white petrolatum control.

For all three allergens, the patch tests were performed on the back using Finn Chambers and Scanpor tape. A 48-h occlusion was used. The readings were carried out on day 2, day 3/4 and day 7 and the reading on day 3 was used for the statistical calculations. Materials and methods are thoroughly described in the previous studies.[3–5] None of the 51 test subjects had active eczema at the time of patch testing with the dilution series.

The threshold concentration was defined as the weakest concentration giving a positive response on day 3 in a continuous line of patch-test reactions starting from the highest concentration. A doubtful reaction (+?) was considered positive in this setting as the test subjects were verified as sensitized and the +? reaction was registered in succession with 1+/2+/3+ reactions.

All 51 test subjects were patch tested with the European baseline series as part of ordinary diagnostics and treatment. If relevant, the test subjects were also tested with additional series. Routine patch-testing methods and materials at the Department of Dermatology and Allergology, Copenhagen University Hospital Gentofte, Denmark have been described previously.[6] Positive reactions to allergens in the European baseline series or supplementary series were counted as additional allergies. A definition of three of more than three contact allergies was chosen for polysensitization, as suggested in two recent reviews.[7,8] The individual combinations of allergens were not chemically/structurally related.

The mean age of the patients was 45·8 years (SD ± 10·4) for the polysensitized group ($n = 13$) and 43·6 years (SD ± 13·4) for the single/double-sensitized group ($n = 38$). An independent samples test showed no difference in mean age between the two groups ($P = 0{\cdot}603$). In the polysensitized group 11 (85%) were female and in the single/double-sensitized group 34 (90%) were female. No difference in sex distribution between the two groups was seen (Fisher's exact test, $P = 0{\cdot}638$).

Statistics

A logistic dose–response model equivalent to the distribution of the threshold doses[9] was estimated from the observed threshold dose–response data by means of asymptotic maximum likelihood methods using specially developed statistical software written in APL (Manugistics Inc., Rockville, MD, U.S.A.). The statistical analysis comprised likelihood ratio tests (χ^2) of goodness of fit, estimation of pairs of parallel logistic threshold dose–response curves, tests of parallelism and calculation of relative sensitivity with 95% confidence intervals (CI). Parallel response vs. $\log_{dose}$ relationships are required for expressing the relative sensitivity or potency as a single number,[9] i.e. the ratio between doses that elicit positive responses in the same fraction of the subjects, e.g. ED_{50} (dose eliciting a reaction in 50% of the subjects). Statistical tests were regarded as significant if $P \leq 0{\cdot}05$.

Independent samples t-test was used to compare age means. The observations were independent and assumption of normality and for equal variances was met.

Results

Nickel sulphate

Twenty nickel-allergic patients were patch tested with a nickel sulphate dilution series. One person did not react to any of the nickel sulphate solutions and was excluded from further analysis. Of the remaining 19 test subjects, 10 had no additional contact allergies, six had one additional contact allergy and three had two additional contact allergies. The lowest threshold concentration observed in the polysensitized group was 0·02%, and in the single/double-sensitized group it was 0·01%. None of the nickel-allergic patients reacted to the ethanol/water control. The parallel dose–response curves for nickel sulphate are shown in Figure 1.

Methyldibromo glutaronitrile

Eighteen MDBGN-allergic subjects were patch tested with a MDBGN dilution series. Of these, four individuals had no additional contact allergies, five had one additional contact allergy and nine had two or more than two additional contact allergies. The lowest threshold concentration observed in the polysensitized group was 0·002%, and in the single/double-sensitized group it was 0·0001%. One of the MDBGN-allergic patients reacted with a few papules, but no erythema and no infiltration, to the ethanol/water control. The parallel dose–response curves for MDBGN are shown in Figure 2.

p-Phenylenediamine

Fifteen PPD-allergic patients were patch tested with a PPD dilution series. One person did not react to any of the PPD solutions and was excluded from further analysis. Of the remaining 14 test subjects, 10 had no additional contact allergies, three had one additional contact allergy and one had three additional contact allergies. The lowest threshold concentration observed in the polysensitized group was 0·05%,

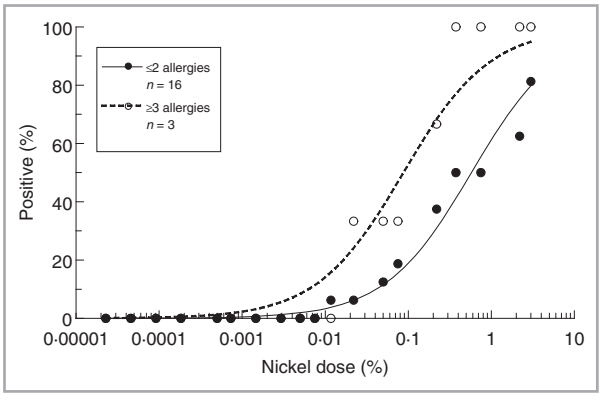

Fig 1. Parallel dose–response curves for nickel sulphate for a polysensitized group and a single/double-sensitized group. The dots represent the percentage of test subjects reacting at a given concentration.

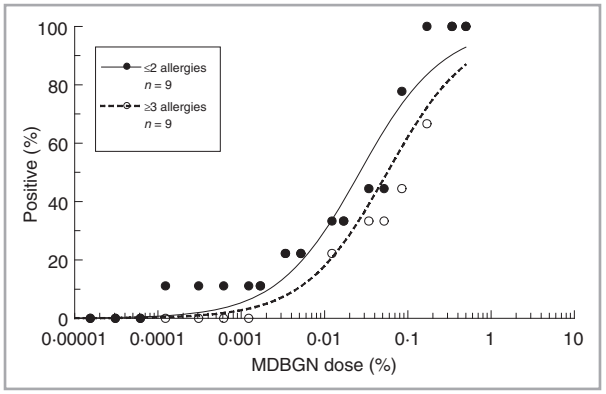

Fig 2. Parallel dose–response curves for methyldibromo glutaronitrile (MDBGN) for a polysensitized group and a single/double-sensitized group. The dots represent the percentage of test subjects reacting at a given concentration.

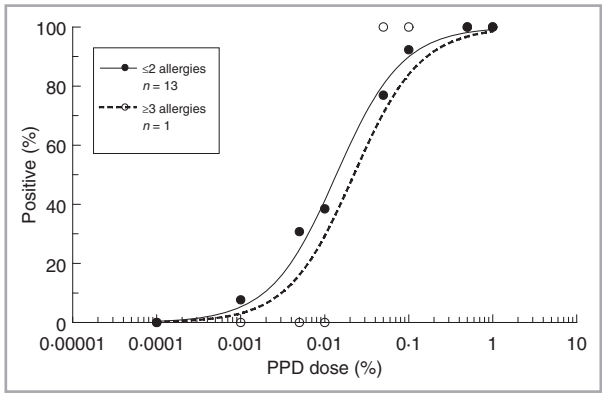

Fig 3. Parallel dose–response curves for *p*-phenylenediamine (PPD) for a polysensitized group and a single/double-sensitized group. The dots represent the percentage of test subjects reacting at a given concentration.

and in the single/double-sensitized group it was 0·001%. None of the PPD-allergic patients reacted to the petrolatum control. The parallel dose–response curves for PPD are shown in Figure 3.

Relative sensitivity

The relative sensitivity was defined as the ratio between the ED_{50} for the single/double-sensitized group vs. the polysensitized group for each allergen separately and for the allergens combined (total test population size 51). The relative sensitivities with 95% CI are shown in Table 1. The relative sensitivities for the three allergens were identical (P = 0·46) which made it possible to summarize the relative sensitivities for each allergen into one combined relative sensitivity. The combined relative sensitivity was 68% (95% CI 19–251) indicating the lowest sensitivity among the polysensitized group; however, there was no significant difference in sensitivity between the single/double-sensitized and polysensitized groups, as the CI included 100%.

Discussion

Under controlled experimental settings using a highly potent allergen (DNCB), polysensitized patients are sensitized by lower induction doses and have greater elicitation responses at every eliciting dose than monosensitized patients and healthy controls.[2] We examined whether or not the elicitation dose–response curve for polysensitized individuals would be displaced to the left compared with a reference group of individuals with one or two contact allergies for allergens to which the patients were already sensitized and, if so, if elicitation thresholds could be estimated beneath which only highly susceptible patients would react.

We analysed the results from testing 51 subjects with dilution series to one of three common allergens to which they were already sensitized. The dose–response curves for the polysensitized group for the allergens MDBGN and PPD were shifted to the right indicating a lower sensitivity compared with the single/double-sensitized group. The dose–response curve of the polysensitized group for the allergen nickel sulphate was shifted to the left indicating a higher sensitivity compared with the single/double-sensitized group. The differences between the dose–response curves were measured in relative sensitivities based on the assumption of parallelism. No significant difference could be demonstrated between the dose–response curves for the polysensitized group vs. the single/double-sensitized group for each of the allergens separately or combined.

The threshold for elicitation is influenced by several exogenous and endogenous factors. Allergen concentration, frequency, duration and occlusiveness of application, body area location of exposure, and simultaneous exposures to other

Table 1 Relative sensitivity[a] for each allergen separately and for the allergens combined

Allergen	Number of test subjects (1–2 contact allergies)	Number of test subjects (≥ 3 contact allergies)	Relative sensitivity (%)	95% Confidence intervals
Nickel	16	3	658	57–31 377
MDBGN	9	9	47	4–293
PPD	13	1	62	2–1334
Total	38	13	68	19–251

Test for same sensitivity: $\chi^2(2) = 1{\cdot}50$, P = 0·46. ED_{50}, dose eliciting a reaction in 50% of subjects; MDBGN, methyldibromo glutaronitrile; PPD, *p*-phenylenediamine. [a]ED_{50} (group with one or two contact allergies)/ED_{50} (group with three or more than three contact allergies).

allergens and irritants, were all controlled in this study. Active inflammation did not affect the results as none of the test subjects had eczema at the time of patch testing. Even though testing with each allergen was performed during series of up to 6 months, seasonal variations probably did not influence the results as the polysensitized and the single/double-sensitized individuals were tested randomly, and not in groups. There was also no age or sex difference between the polysensitized and single/double-sensitized groups which could affect the results.

The test subjects were included based on a positive patch-test response to the allergen of interest. The test subjects had been referred to our Department of Dermatology and Allergology in need of diagnosis and treatment of a skin disorder. The circumstances surrounding sensitization for the individual test subjects were not standardized and the test subjects consequently expressed heterogeneity regarding real-life conditions of exposures leading to sensitization. The magnitude of the elicitation response is dependent on the induction dose: the greater the induction dose, the greater the elicitation response.[10] Heterogeneity between cases and controls regarding conditions of exposure leading to sensitization may have influenced the strength and threshold of the elicitation response which may explain why no difference could be detected. However, this setting is reflecting the exposure situations in reality.

DNCB sensitization in polysensitized individuals divided according to exposure into a group with high occupational exposure and a group with ubiquitous baseline exposure showed that only the latter group expressed a decreased sensitization threshold.[2] The polysensitized individuals in our study were not subdivided according to specific conditions leading to polysensitization, a task that would be difficult and not realistic. Only one subgroup of polysensitized individuals expressed an increased sensitivity, and furthermore this was a subgroup without intense exposure as a cause of polysensitization; therefore, heterogeneity, not only between cases and controls regarding conditions of exposures, but also within our used case group, may also be the reason why no difference was detected.

Allergic mechanisms are allergen specific which may also explain the different outcomes when using MDBGN, PPD and nickel sulphate in contrast to DNCB.

The grouping of patients with one or two contact allergies vs. patients with three or more than three contact allergies is based on a previous recommendation. This seemed reasonable when focusing on patients with suspected increased reactivity, as patients with three or more than three contact allergies appear more frequently then would be expected by chance[1,2] and pairs of contact allergies can often be ascribed to concomitant exposure and cross-reactivity, in contrast to triplets of contact allergies.[7] Increased reactivity may be a graded phenomenon where the reactivity increases with increasing number of contact allergies.[2] The choice to combine individuals with one or two contact allergies in the reference group could, therefore, have obliterated any true but small difference. The statistical calculations were, however, also performed for a monosensitized group vs. a group with two or more than two contact allergies, which also almost equalized the number of test subjects in each study group, but it did not change the results. No significant differences were detected.

No increased sensitivity could be demonstrated in polysensitized subjects compared with individuals with one or two contact allergies. The dose–response relationships were based on data from small test groups which reduces the precision of the calculated relative sensitivities. The polysensitized test group was particularly small and the number of test subjects in each study group diverged. It is possible that a difference in relative sensitivity between the two groups truly exists but it was not strong enough to appear in this experimental setting and with the number of test subjects used.

Acknowledgments

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CONTACT DERMATITIS

Characterization of the polysensitized patient: a matched case–control study

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Background: Polysensitization (≥ 3 contact allergies) may be regarded as a special entity in patients with contact allergies. However, this group of polysensitized patients is poorly characterized. Filaggrin mutations are associated with atopic eczema and lead to impaired skin barrier which may predispose to contact allergy. Therefore, it is of interest to consider atopic eczema and contact allergies, especially in patients with multiple allergies.

Objective: To characterize polysensitized patients regarding occurrence, duration and course of dermatitis, and examine potential risk factors for polysensitization, including atopic eczema.

Methods: A questionnaire case–control study of 562 polysensitized and 1124 single/double-sensitized individuals was performed.

Results: The results show that 45% of polysensitized and 31% of single/double-sensitized patients had or had had atopic eczema, and atopic eczema was identified as a risk factor for polysensitization. Patients with leg ulcer constituted only a minor part of the polysensitized group and leg ulcers were not identified as a risk factor for polysensitization in this study. The influence of contact allergies on duration and course of disease diverged between the group of patients with atopic eczema and the group without atopic eczema.

Conclusion: Patients with atopic eczema were overrepresented in the group of polysensitized patients and polysensitized patients should be viewed in the light of occurrence or lack of atopic eczema.

Key words: polysensitization; contact allergy; multiple contact allergies; atopic eczema; duration of disease; persistency of symptoms; leg ulcers.

Conflicts of interest: The authors have declared no conflicts.

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Polysensitized patients may be defined as patients with three or more contact allergies to environmental substances (1, 2). These individuals show greater elicitation responses than mono-allergic and healthy controls when exposed to dinitrochlorobenzene (3) but not when exposed to p-phenylenediamine, nickel sulphate and methyldibromo glutaronitrile (4). They are primarily women and the risk of polysensitization increases with age (5). Some allergens have been identified as potential risk factors for polysensitization but an overall common denominator for these allergens are lacking (6). Occurrence of leg ulcers and stasis dermatitis has been known risk factors for polysensitization for long (7). General perceptions regarding course, severity and duration of disease in polysensitized patients exist but have not been studied systematically.

Recently, filaggrin mutations have been linked to atopic eczema and ichthyosis vulgaris (8, 9). Filaggrin mutations lead to impaired skin barrier with increased penetration of environmental substances which raises the possibility that filaggrin mutations might also predispose to allergic contact sensitization (10). The association between polysensitization and atopic eczema was examined in this study. A further aim was to characterize the polysensitized

patient regarding occurrence, duration and course of dermatitis and examine potential risk factors for polysensitization.

Materials and Methods

Study population and design

During a 20-year period, 14 998 patients were patch tested with the European Baseline Series (23 allergens) at the Department of Dermato-Allergology, Gentofte University Hospital, Denmark. Patch testing was done with Finn Chambers® and TROLAB® patch test allergens applied to the upper back for 2 days. Readings were done on D2, D3/D4 and D7 according to the ICDRG (11). A 1+, 2+ and 3+ reading was interpreted as a positive response. Patch test method, interpretations of patch test reactions and results were previously reported in detail (5). A total of 759 (5.1%) patients were polysensitized. Of these, 562 individuals were still alive, had not emigrated and could be located for inclusion in this study. The 562 polysensitized individuals were matched for sex, age ± 2 years and time of patch test ± 24 months in a 1:2 order with individuals with 1–2 contact allergies, making a total study population of 1686 individuals. Matching was performed using the computer programme SQL Query Analyzer® version 8.00.194 (Microsoft Corporation, Redmond, WA, USA) which listed all controls in the database who matched the subject case according to the chosen boundaries for age, sex and patch test year. Two controls, still alive and living in Denmark and with the closest match on age but still within the chosen boundaries for patch test year, were chosen for each case. For one control, age diverged by 3 years and for six controls, time of patch test diverged by 26–48 months.

Questionnaire

The 1686 participants received a postal questionnaire. A second questionnaire was posted after 5 weeks to the participants that did not respond to the first questionnaire. The questionnaire was initially tested in 4 nurses and 1 information technology consultant with atopic eczema to identify any major problems related to structure, wording and response categories. This was succeeded by a pilot test and retest in a total of 40 consecutive outpatients undergoing patch testing. In six patients, questions and answers eligible to be checked against the patient records were performed. The questionnaire consisted of 70 items; mainly fixed-response questions but also some open-ended questions where needed. The items covered aspects of self-reported dermatitis, work, education, contact allergies and patch testing, general health and other skin diseases, multiple chemical sensitivities and dermatitis in straight-line relatives. Questions relevant to this paper included self-reported dermatitis, year of onset of dermatitis, year of last dermatitis eruption, outbreak frequency, atopic eczema, other skin diseases and education.

Definitions

A diagnosis of dermatitis was defined as 'yes' to the question 'Have you ever had dermatitis?'. The UK Working Party's Diagnostic Criteria question-only version, were used to identify patients with atopic eczema (12). A diagnosis of other skin diseases was defined as 'yes' to the question 'Have you ever had one of the following skin diseases: psoriasis, itch without visible skin lesions, urticaria, leg ulcers, or other skin disease, please specify'.

The duration of disease was measured in years by subtracting the debut year from the year where the last dermatitis episode occurred. The duration of disease, therefore, measures the total duration between first and last dermatitis episode regardless of intermittent dermatitis-free episodes. Age at debut was measured in years by subtracting the birth year from the year of debut of dermatitis.

Patients were asked whether or not the dermatitis occurred intermittently and if so, how much of the time between first and last dermatitis episode they had been free of dermatitis. Four options were given, if they had been free of dermatitis for more than half of the time, about half of the time, less than half of the time, or none of the time (= persistent dermatitis) between first and last dermatitis episode.

Educational level was based on years of education. Six groups of education level were used: lowest level (≤10 years of education), low level (11–12 years of education), basic level (13–14 years of education), medium level (15–16 years of education), high level (>17 years of education), and education level unknown/education ongoing. The Danish Educational Nomenclature, developed by the organization Statistics Denmark and Danish Ministry of Education, was used to classify each specific education into educational levels.

Responders

1120 returned the questionnaire corresponding to a response rate of 66.4%. 70.1% of the polysensitized and 64.6% of the single/double-sensitized individuals answered the questionnaire (χ^2, $p < 0.05$). Missing data ranged from 0.5% for self-reported

dermatitis to 6.2% for duration of disease. Comparison of mean age and sex distribution between the polysensitized and single/double-sensitized responders is illustrated in Table 1. The same percentage of polysensitized and single/double-sensitized responders were patch tested each year between 1985 and 2005 (results not shown).

Non-responders

566 individuals did not return the questionnaire (33.6%). Comparison of mean age, sex distribution and number of polysensitized individuals between responders and non-responders is given in Table 2. More individuals patch tested in the recent years responded to the questionnaire compared with the first years (figure 1), (χ^2_{trend}, $p < 0.001$). More responders compared with non-responders had contact allergy to potassium dichromate, fragrance mix I and quaternium-15 (results not shown) which became non-significant after adjustment for multiple testing.

Statistics

The statistical analyses were performed in SPSS® software version 15.0 (SPSS Inc., Chicago, IL, USA).

Comparisons of frequencies and sensitivity rates were made using the χ^2 test and evaluation of trends with χ^2 for trend. Comparison of mean age was done using the independent samples T test. Assumptions of normality and independence were met. Assumption of equal variances was met for comparison of mean age for cases and controls in the responder group but not met for comparison of mean age between non-responders and responders. Comparison of median age of debut and duration of disease between two groups was done using the Mann Whitney test.

Because of multiple testing when comparing sensitivity rates for each allergen in the European Baseline Series the p-value was adjusted according to the method of Bonferroni so that a p-value below 0.002 was regarded as significant. For the remaining calculations a p-value below 0.05 was regarded as significant.

Table 1. Comparison of single/double-sensitized and polysensitized responders

	Responders ($n = 1120$)		
	Single/double-sensitized $N = 726$ (64.8%)	Polysensitized $N = 394$ (35.2%)	TEST, P VALUE
Age (years)*	47.5, SD±14.4	47.8 years SD±14.8	T test, $p = 0.767$
Female sex#	589 (81.1%)	326 (82.7%)	χ^2, $p = 0.51$

*Mean ± standard deviation
#Number (percentage)

Table 2. Comparison of responders and non-responders

	Responders $n = 1120$ (66.4%)	Non-responders $n = 566$ (33.6%)	Test, p value
Age (years)*	47.6 ±14.5	49.2 ± 15.9	T test, $p = 0.052$
Female sex#	915 (81.7%)	453 (80.0%)	χ^2, $p = 0.41$
Polysensitization#	394 (35.2%)	168 (29.7%)	χ^2, $p = 0.024$

*Mean ± standard deviation
#Number (percentage)

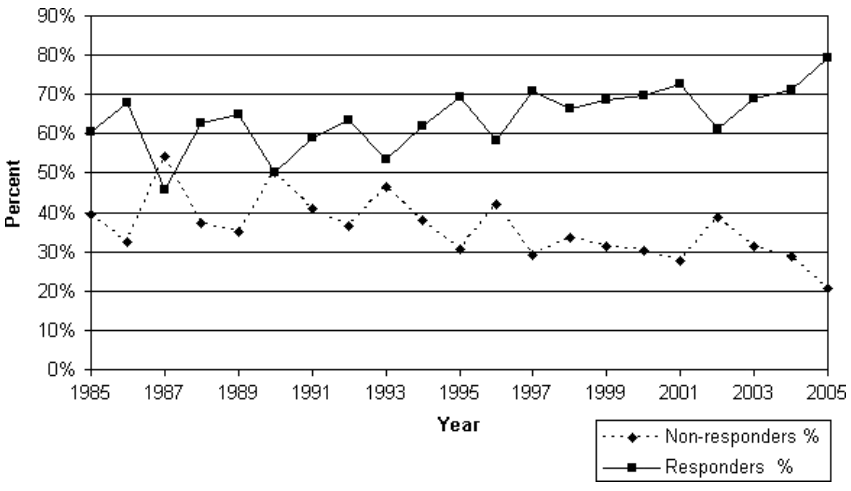

Fig. 1. Study subjects divided according to patch test year and status of response.

Risk factors for polysensitization were examined by multivariate regression analyses. Three logistic regression analyses with polysensitization versus single/double-sensitization as dependent variable; atopic eczema, education level, duration of disease, outbreak frequency and leg ulcers as explanatory variables and sex, age, and time of patch test as co-factors were performed. The analyses were based on, respectively, a population consisting of all respondents, a population of patients with atopic eczema and a population of patients without atopic eczema.

Results

Dermatitis

The majority of responders had suffered from dermatitis at some point in time in their lives (983 (88.2%)). 93.4% (365) of the polysensitized responders and 85.5% (618) of the single/double-sensitized responders had or had had dermatitis (χ^2, $p < 0.001$).

Atopic eczema

397 (36.0%) individuals had or had had atopic eczema. 31.0% (221) among the single/double-sensitized and 45.1% (176) among the polysensitized group had atopic eczema (χ^2, $p < 0.001$).

Frequency of the different patch test readings; doubtful reaction (+?), 1+, 2+ and 3+ positive reaction, follicular reaction and irritant reaction was compared between patients with atopic eczema and patients without atopic eczema. Patients with atopic eczema had a higher frequency of irritant reactions for potassium dichromate readings on D3 and D7 compared with patients without atopic eczema (χ^2, results not shown) but not for any other irritant reaction readings for the remaining 22 allergens. No differences in frequency of 1+, 2+ or 3+ positive reactions was detected between patients with and without atopic eczema, but patients with atopic eczema had a higher frequency of +? reactions for neomycin D3, benzocaine D2 and D3, formaldehyde D2, mercapto mix D3, methylchloroisothiazolinone/methylisothiazolinone D7, mercaptobenzothiazole D3, and for sesquiterpene lactone mix D7 readings (results not shown). Furthermore, patients with atopic eczema had a higher frequency of follicular reactions for p-phenylenediamine D3, quaternium-15 D7, and sesquiterpene lactone mix D2 readings. Doubtful (+?) and follicular reactions were regarded as negative reactions.

Other skin diseases

No overrepresentation of self-reported psoriasis, itch without visible skin lesions, or urticaria was found in the polysensitized group compared with the single/double-sensitized group (χ^2, respectively, $p = 0.94$, $p = 0.94$, $p = 0.45$). Also, no overrepresentation of self-reported leg ulcers was found in the polysensitized group (8.6%—33 individuals) compared with the single/double-sensitized group (6.7%—47 individuals) (χ^2, $p = 0.27$).

Educational level

Distribution of patients according to educational level and number of contact allergies is illustrated in Table 3. None of the six educational levels were overrepresented among the polysensitized group compared with the single/double-sensitized group (χ^2-tests, results in Table 3).

Duration of disease

It was possible to calculate the duration of disease in 928 individuals. 2.0% (19) had duration of dermatitis of <1 year. The duration of dermatitis ranged from 1 to 84 years with a rightly skewed distribution.

Duration of dermatitis was estimated for a group with and without atopic eczema. Overall median duration of disease among polysensitized patients without atopic eczema was 22.0 years [inter-quartile range (IQR) 20.75] and among single/double-sensitized patients without atopic eczema 15.5 years (IQR 22.75) (Mann Whitney, $p = 5.7 \cdot 10^{-5}$). The overall median duration of disease among polysensitized with atopic eczema was 33.0 years (IQR 23.25) and 29.0 years (IQR 25.0) among single/double-sensitized patients with atopic eczema (Mann Whitney, $p = 0.11$).

Table 3. Distribution of patients according to educational level and number of contact allergies

Educational level	1-2 Contact allergies	≥ 3 Contact allergies	X- square test, p value
Lowest level (≤ 10 years)	11.6% (84)	15.0% (59)	> 0.1
Low level (11–12 years)	38.2% (277)	35.3% (139)	> 0.2
Basic Level (13–14 Years)	10.5% (76)	13.7% (54)	> 0.1
Medium level (15–16 years)	21.3% (155)	21.6% (85)	> 0.2
High level (≥ 17 years)	9.1% (66)	7.4% (29)	> 0.2
Educational level unknown/ education ongoing	9.4% (68)	7.1% (28)	> 0.1

Table 4. Duration of disease estimated for a group with atopic eczema and without atopic eczema according to number of contact allergies

Status of atopic eczema	Number of sensitizations	Median duration (inter-quartile range)
Atopic eczema	1	29.0 (24.25)
	2	33.0 (27.5)
	3	32.0 (24.0)
	4	37.0 (30.75)
	≥ 5	32.0 (20.5)
Not atopic eczema	1	15.0 (22.0)
	2	17.0 (26.0)
	3	18.0 (17.0)
	4	25.0 (25.0)
	≥ 5	34.0 (23.0)

For the group without atopic eczema the median duration of disease increased with the number of contact allergies, while a similar relationship was not seen for the group with atopic eczema (Table 4).

Outbreak frequency

Occurrence of dermatitis is often intermittent. Figure 2A and 2B illustrates for a polysensitized and single/double-sensitized group divided according to status of atopic eczema, the number of individuals who reported persistent dermatitis, dermatitis for less than half of the time between first and last dermatitis episode, dermatitis for about half of that time and dermatitis for more than half of the time between first and last dermatitis episode.

In the group without atopic eczema, there was no difference between occurrence of poly- and single/double-sensitized individuals who reported dermatitis for the entire period, less than half of the period, for half of the period, or for more than half of the period from first to last dermatitis episode.

A larger proportion of patients with atopic eczema and with polysensitization (38.5%) reported dermatitis for the entire period compared with the group of patients with atopic eczema and with single/double-sensitization (24.9%) (χ^2, $p < 0.01$). Inversely, a larger proportion of the patients with atopic eczema and with single/double-sensitization (32.4%) reported dermatitis for less than half of the period compared with the polysensitized group (23.0%) (χ^2, $p < 0.05$).

Age at debut of dermatitis

The median age at debut was 31.0 years (IQR 30.0) and 29.5 years (IQR 27.0), respectively, for the single/double-sensitized and the polysensitized group without atopic eczema (Mann Whitney, $p = 0.019$). Median age at debut did not differ between the single/double-sensitized (18.0 years, IQR 28.0) and polysensitized groups with atopic eczema (18.0 years, IQR 26.0, Mann-Whitney $p = 0.473$.)

Risk factors for polysensitization

Risk factors for polysensitization were examined by multivariate regression analyses. Atopic eczema was significantly positively associated with polysensitization, see Table 5. Duration of disease showed significant associations with polysensitization in the total respondent population and in the population of patients without atopic eczema. Outbreak frequency was significantly associated with polysensitization in the atopic eczema group. Leg ulcers and education level were not associated with polysensitization.

Discussion

The results suggest that polysensitized individuals should be viewed in the light of occurrence or lack of atopic eczema. In the following, three different terms were used: *duration of disease* which corresponds to the length of disease in years regardless of outbreak frequency; and *persistent and intermittent dermatitis* which corresponds to the outbreak frequency.

Polysensitized individuals with atopic eczema were characterised by having more persistent eczema compared with single/double-sensitized individuals with atopic eczema. Nearly 40% of polysensitized individuals with atopic eczema had persistent dermatitis compared with about 25% of the single/double-sensitized group. Regarding duration of dermatitis, both single/double-sensitized and polysensitized individuals with atopic eczema had long duration of eczema, respectively 29 and 33 years on average, which was not statistically significantly different. It suggests that contact allergies do not influence the duration of dermatitis for patients with atopic eczema which is in agreement with one other study (13).

54.9% of the polysensitized group did not have atopic eczema and they did not differ regarding course of disease from single/double-sensitized individuals without atopic eczema. About 20% in both groups had persistent dermatitis and the rest intermittent dermatitis. However, the polysensitized patients without atopic eczema had longer duration of dermatitis compared with single/double-sensitized individuals without atopic eczema. Contact allergy has previously been shown to influence the prognosis of dermatitis negatively in a population with hand eczema (14, 15). Even though age at onset diverged between the poly- and single/double-sensitized groups without atopic eczema, the absolute difference in age between the two groups was

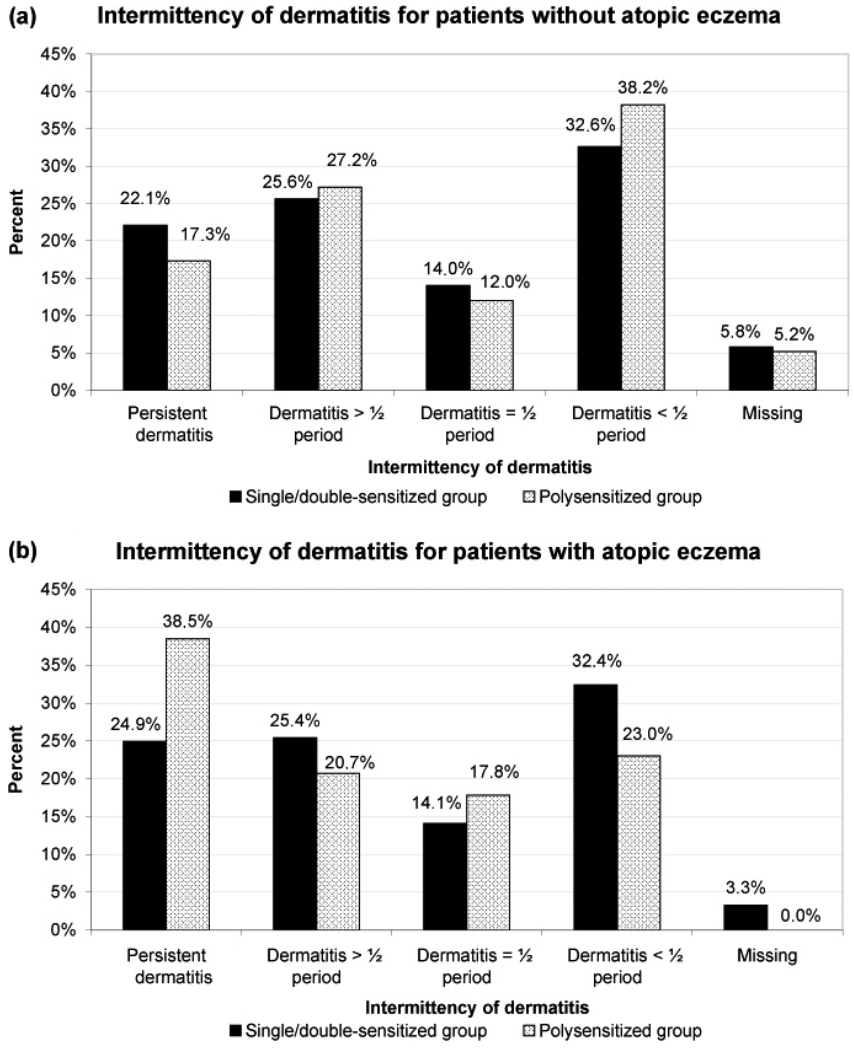

Fig. 2. (a) Intermittency of dermatitis for polysensitized and single/double-sensitized individuals without atopic eczema. (b) Intermittency of dermatitis for polysensitized and single/double-sensitized individuals with atopic eczema.

small and does not explain the difference in duration of disease.

The positive association between duration of disease and number of sensitizations in the group without atopic eczema may be explained by difficulty in avoidance of contact with the relevant allergens due to the many allergies with multiple exposure routes, or reflect that long duration of skin disease with impaired skin barrier predisposes to polysensitization. Whether or not the long duration of disease is the cause or consequence of polysensitization cannot be answered in this retrospective design. A higher rate of irritant contact dermatitis among the polysensitized group cannot be ruled out and may have contributed to maintenance of disease and predisposed to polysensitization by increasing risk of sensitizations (16). The ability to avoid allergens is dependent on understanding of diagnosis and knowledge of patch test results and effectiveness of information depended on education level, ethnic background and age (17, 18). Education level and age did not show any discrepancy between the polysensitized and single/double-sensitized group in this study. Long duration of disease may be the consequence of and not the cause of polysensitization; therefore, no obvious risk factors were found in the sub-group of polysensitized individuals without atopic eczema.

Other factors important for persistence of dermatitis include age and sex, for which the groups in this study were matched, specific occupations, duration of symptoms before diagnosis and type of allergy (19, 20) and how widespread the dermatitis was at initial examination in patients with hand eczema (14). It cannot be ruled out that similar factors are important in this cohort and differed between cases and controls in the study.

Atopic eczema and polysensitization

The prevalence estimate of 36% of atopic eczema in this study corresponds well with prevalences of atopic eczema in other dermatitis populations ranging from 16.7% to 46% (21–23). Nearly half

Table 5. Logistic regression analyses with polysensitization as dependent variable and atopic eczema, educational level, duration of disease, outbreak frequency and leg ulcers as explanatory variables. Three logistic regression analyses are presented based on a population of, respectively, all respondents, patients with atopic eczema and patients without atopic eczema

	Total population*		No atopic eczema*		Atopic eczema*	
Explanatory variables	No.	OR (95% CI)	No.	OR (95% CI)	No.	OR (95% CI)
No atopic eczema	542	Reference	—	—	—	—
Atopic eczema	354	**1.43 (1.06–1.93)**	—	—	—	—
Educational level						
< 10 yrs	108	Reference	64	Reference	42	Reference
11–12 yrs	344	0.70 (0.44–1.11)	203	0.55 (0.30–1.01)	139	0.97 (0.47–2.00)
13–14 yrs	106	0.93 (0.53–1.64)	74	0.69 (0.33–1.42)	31	1.09 (0.41–2.87)
15–16 yrs	202	0.69 (0.42–1.14)	110	0.51 (0.26–1.01)	90	1.03 (0.47–2.22)
> 17 yrs	81	0.68 (0.37–1.28)	58	0.50 (0.22–1.10)	23	1.01 (0.35–2.92)
Duration of disease						
0–9 yrs	191	Reference	150	Reference	36	Reference
10–19 yrs	208	**2.20 (1.40–3.47)**	150	**2.20 (1.28–3.76)**	57	2.10 (0.84–5.30)
20–29 yrs	157	**2.54 (1.56–4.12)**	87	**3.34 (1.82–6.14)**	70	1.79 (0.74–4.35)
30–39 yrs	156	**2.54 (1.56–4.15)**	81	**2.19 (1.16–4.13)**	74	**2.84 (1.16–6.91)**
>= 40 yrs	192	**2.72 (1.67–4.42)**	74	**2.87 (1.49–5.53)**	117	2.29 (0.89–5.33)
Outbreak frequency						
Persistent eczema	223	Reference	112	Reference	111	Reference
Dermatitis > 1/2 of period	225	0.83 (0.56–1.23)	140	1.32 (0.76–2.30)	79	**0.54 (0.30–0.99)**
Dermatitis = 1/2 of period	132	0.75 (0.48–1.19)	75	0.92 (0.47–1.80)	57	0.68 (0.35–1.33)
Dermatitis < 1/2 of period	297	0.79 (0.55–1.15)	194	1.43 (0.84–2.42)	102	**0.43 (0.25–0.77)**
No leg ulcers	833	Reference	504	Reference	321	Reference
Leg ulcers	71	1.13 (0.68–1.90)	38	1.08 (0.52–2.24)	33	1.05 (0.49–2.28)

OR = odds ratio,
CI = confidence interval,
*adjusted for sex, age, and patch test year.

of our polysensitized patients (45%) and 31% of the patients with 1–2 contact allergies suffered from atopic eczema. In a recent German study, 38.4% of all polysensitized individuals and 37.8% of single/double-sensitized individuals had atopic eczema (24). Some decades ago patients with atopic eczema constituted a minor part of patch test populations (25) whereas atopic eczema today is more common in patch test populations. The frequency of atopic eczema has increased over the past decades which cannot be explained by genetics (26). The suspected triggers of this increase have focussed on environmental factors (27). This increase in frequency of atopic eczema is reflected in the composition of contact allergy cohorts today.

It can be argued, that individuals with atopic eczema have a higher skin reactivity (28–30) and therefore more easily develop irritant and doubtful reactions. Frequent irritant and doubtful reactions hold the potential for mis-interpretations of patch test reactions as false-positive reactions which would increase the rate of individuals with atopic eczema and with contact allergies and multiple contact allergies. We found a higher frequency of irritant reactions for potassium dichromate readings on D3 and D7 but not for any other standard allergens. Some higher frequencies of follicular and doubtful reactions were also recorded for patients with atopic eczema compared with patients without atopic eczema. The significant differences in the specific reactions occurred in a non-systematic way and did not result in a generally higher rate of positive readings among patients with atopic eczema. Therefore, we assess that an overrepresentation of irritant reactions does not cause a systematic bias in the prevalence estimates of contact allergies among patients with atopic eczema.

Contact allergy among patients with atopic eczema can be explained by impaired skin barrier with increased allergen absorption combined with long-term treatments with frequent exposures to various substances which increase the risk of contact sensitization. This is supported by a positive relationship between severity and duration of atopic eczema and frequency of contact sensitizations (31, 32) and a higher sensitivity rate among non-healed compared with healed patients with atopic eczema (33). Even though patients with atopic eczema experimentally have a diminished ability to develop contact allergies (34, 35), contact allergies are not infrequent events among patients with atopic eczema as 17.1–54% of patients with atopic eczema have contact allergy (13, 22, 36). Patients with atopic eczema had an increased risk of polysensitization compared to non- atopic eczema patients. Atopic eczema is associated with filaggrin loss-of-function mutations (8) and these mutations are also suspected to be associated with contact allergy (37, 38). It is possible that our finding regarding atopic eczema and polysensitization is confounded by filaggrin mutations.

Leg ulcers and polysensitization

Polysensitized patients with leg ulcers were not overrepresented and leg ulcers did not constitute a risk factor for polysensitization. Patients with leg ulcers have been a high-risk population for development of polysensitization as 68–75% were sensitized, and 51–57% were polysensitized (7, 39). New topical treatments with low sensitizing potency developed in the recent years may have diluted the previously seen high frequency of polysensitization among patients with leg ulcer. The higher frequency of atopic eczema in the polysensitized group may also have diluted any difference in leg ulcer frequency between the poly- and single/double-sensitized group (24, 36).

Potential sources of bias

Both cases and controls were drawn from a hospital population minimizing discrepancy in selection factors between the two groups and also decreasing the likelihood of non-response and recall bias. They constitute the patients with most severe dermatitis due to selective referral to hospital departments and the results may not be relevant for other dermatitis populations. Matching for age and sex was performed to neutralize them as confounding factors. Matching for age and time of patch tests minimized differential recall bias.

We cannot entirely eliminate recall bias. Relapses may be confused with debuts and occurrence of dermatitis may not be correct especially if the disease occurred many years ago. Much of such recall bias is likely non-differential.

Mild cases with short duration of disease and patients with disease ending many years ago may not remember their disease and may be more likely not to respond to the postal questionnaire. If they were missed, it may have shifted the duration of disease for both cases and controls toward longer duration of disease or toward the null hypothesis if this was more pronounced for the single/double-sensitized group.

Conclusion

Polysensitized patients suffer from dermatitis, nearly every other suffers from atopic eczema. Long duration of disease was associated with polysensitization but it cannot be determined in this cross-sectional design whether long duration of disease was a cause or consequence of polysensitization. Atopic eczema was the only identified risk factor for polysensitization. Leg ulcers and educational level did not seem to be risk factors for polysensitization.

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Sites of dermatitis in a patch test population: hand dermatitis is associated with polysensitization

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Summary

Background Sites of dermatitis in larger series of contact allergic patients are rarely reported. Increased risk of polysensitization has been linked only to stasis dermatitis and leg ulcers. However, a large proportion of polysensitized individuals may have dermatitis in other skin areas.

Objectives To examine the site of dermatitis at time of first appearance in contact allergic individuals with special focus on the distribution of dermatitis in polysensitized individuals and to examine if widespread dermatitis is more frequent in polysensitized than in single/double-sensitized patients.

Methods A matched case–control study was carried out including 394 polysensitized and 726 single/double-sensitized patients who responded to a postal questionnaire. All subjects were recruited from a hospital patch test population.

Results The hands were the most frequent and the anogenital region was the least frequent skin area affected with dermatitis. Dermatitis on the hands/wrists [odds ratio (OR) 1·58], in the armpits (OR 1·56) and on the back (OR 1·91) was positively associated with polysensitization. The hands were the only skin area with dermatitis which maintained the association to polysensitization in two subpopulations consisting of, respectively, individuals with and without atopic eczema. Dermatitis on the scalp was negatively associated with polysensitization (OR 0·66) primarily for individuals without atopic eczema. The dermatitis did not seem to be more widespread in polysensitized compared with single/double-sensitized patients.

Conclusions Special awareness in patients with hand dermatitis seems justified either to prevent development of multiple contact allergies or to document polysensitization as an aetiological factor.

Regional contact dermatitis often leads to suspicion of causative exposure, e.g. stasis dermatitis and contact allergies to ulcer bandage materials, scalp to hair dyes, armpits to deodorants, anogenital dermatitis to topical medicaments, earlobes to nickel-releasing jewellery, etc. It is likely that dermatitis in certain skin regions is also associated with increased risk of multiple contact allergies (polysensitization). Only stasis dermatitis in the lower legs has so far been associated with polysensitization.[1] Hand dermatitis has been reported to occur less frequently in polysensitized than in monosensitized patients.[2]

One study showed an increased occurrence of generalized dermatitis in polysensitized patients compared with monosensitized patients,[2] and in another study polysensitized individuals who had been sensitized and experimentally exposed to dinitrochlorobenzene showed an increased reactivity,[3] which may result in more severe dermatitis but not necessarily in more widespread dermatitis.

The aim of this study was to examine a population of polysensitized patients with dermatitis from a hospital patch test population and compare this group with a group of single/double-sensitized patients with regard to (i) site of dermatitis at time of first appearance, and (ii) extent of distribution of dermatitis. Polysensitization was defined as three or more contact allergies.[4,5]

Materials and methods

Study population

From 1985 to 2005, 14 998 patients were patch tested at the Department of Dermato-Allergology, Gentofte Hospital,

Denmark. The patch test method and readings have previously been described in detail.[6] All patients were tested with the European baseline series but not necessarily to additional series. Counting of the number of sensitivities was, therefore, based on reactions to the European baseline series only. All individuals who had three or more positive reactions were classified as polysensitized. A total of 562 individuals who were polysensitized, and were still alive, had not emigrated and could be located at the time of the present study were included in the study. Each polysensitized patient was individually matched with two patients with one or two positive reactions (single/double-sensitized). The total study population, therefore, included 1686 individuals. The matching parameters were sex, age and time of the patch test. Matching procedures have previously been described.[7] All participants received a postal questionnaire. Another questionnaire was posted after 5 weeks to the individuals who did not respond to the first questionnaire. The questionnaire was returned by 1120 subjects, corresponding to a response rate of 66·4%; 70·1% (394) of the polysensitized individuals and 64·6% (726) of the single/double-sensitized individuals answered the postal questionnaire. Patients with atopic eczema (AE) were over-represented among polysensitized individuals in this cohort.[7] Dropout analysis was previously reported.[7]

Questionnaire and definitions

The questionnaire consisted of 70 items: mainly fixed-response questions but also some open-ended questions where needed. The items covered aspects of self-reported dermatitis, work, education, contact allergies and patch testing, general health and other skin diseases, multiple chemical sensitivities and dermatitis in straight-line relatives. Questions relevant to this paper included self-reported dermatitis, AE and site of dermatitis at time of first appearance.

The responders were asked where the dermatitis was located at the time of first appearance. Nineteen different skin sites could be documented for each patient as the body surface was divided into the following sites: scalp, periorbital region, periauricular region, perioral region, remaining part of face, neck, shoulders, armpits, cubital folds, arms excluding hands, hands and/or wrists, chest, back, stomach, buttocks, popliteal folds, legs excluding feet, feet and/or ankles, and the anogenital region. No restrictions were made regarding the number of skin areas marked as affected with dermatitis at time of first appearance; a minimum of one and a maximum of 19 areas were reported.

A diagnosis of dermatitis was defined as 'yes' to the question, 'Have you ever had dermatitis?'. The U.K. Working Party's Diagnostic Criteria, question-only version, were used to identify patients with AE.[8]

The questionnaire was evaluated in four nurses and one IT consultant with AE, and with a pilot test and retest in a total of 40 consecutive outpatients undergoing patch testing. In the pilot test, 16 response categories were represented in the question regarding localization of dermatitis at time of first appearance. The cubital and popliteal folds regions were included in the arms and legs regions, and the buttocks were combined with the anogenital region. The pilot test revealed that the initial 16 response categories were not sufficient and in the retest 19 response categories were represented where the cubital and popliteal folds were separated from, respectively, the arms and the legs, and the buttocks and anogenital region were split into separate categories. In the retest the questionnaire functioned well and was considered easy to understand and to answer by the test subjects.

Statistics

The statistical analyses were performed in SPSS® software version 15.0 (SPSS Inc., Chicago, IL, U.S.A.). A minor part of the total population reported never suffering from dermatitis and were excluded from further analysis.[7]

Three logistic regression analyses were performed to test the association between polysensitization and specific skin areas affected with dermatitis at first appearance. One model was based on the total population and two models were based on, respectively, patients with and without atopic eczema, as a characteristic distribution pattern of dermatitis is observed in AE and patients with AE were over-represented among the polysensitized individuals.[7] In each model, polysensitization vs. having one to two contact allergies was used as a dependent outcome and all 19 skin areas were included as independent variables. Age, sex, AE and patch test year were included as cofactors for the model including the total population, and age, sex and patch test year were included as cofactors for the two models concerning subpopulations according to AE status.

The Mann–Whitney test was used to compare the median number of skin areas affected at the time of first appearance because the data were skewed.

Correlations between the different skin areas were examined by multiple 2×2 tables for two different strata – individuals with and without AE – and the hypothesis was tested with χ^2 tests. Because of multiple testing the P-value was adjusted according to the method of Bonferroni so that $P \leq 2{\cdot}9 \times 10^{-4}$ was regarded as significant. For the remaining calculations $P < 0{\cdot}05$ was regarded as significant.

Results

Site of dermatitis at time of first appearance

The frequency of each skin area affected with dermatitis at first appearance for, respectively, polysensitized and single/double-sensitized individuals with and without AE is illustrated in Table 1. The hand and wrist was the most frequent and the anogenital region was the least frequent skin area affected for all four groups.

Using logistic regression analyses, we tested whether or not specific skin areas affected with dermatitis at first appearance were associated with polysensitization (Table 2). Dermatitis on the hands/wrists, in the armpits and on the back was associated positively with polysensitization with odds

Table 1 Frequency of dermatitis at first appearance in 19 different skin areas

	No atopic eczema		With atopic eczema	
Skin area	Single/double-sensitized individuals (n = 393), n (%)	Polysensitized individuals (n = 187), n (%)	Single/double-sensitized individuals (n = 212), n (%)	Polysensitized individuals (n = 173), n (%)
Scalp	62 (15·8)	15 (8·0)	45 (21·2)	35 (20·2)
Periorbital region	49 (12·5)	26 (13·9)	50 (23·6)	30 (17·3)
Periauricular region	47 (12·0)	22 (11·8)	34 (16·0)	20 (11·6)
Perioral region	28 (7·1)	15 (8·0)	24 (11·3)	20 (11·6)
Remaining part of face	51 (13·0)	27 (14·4)	37 (17·5)	35 (20·2)
Neck	47 (12·0)	24 (12·8)	42 (19·8)	42 (24·3)
Shoulders	25 (6·4)	12 (6·4)	22 (10·4)	19 (11·0)
Armpits	37 (9·4)	24 (12·8)	33 (15·6)	36 (20·8)
Cubital folds	35 (8·9)	16 (8·6)	73 (34·4)	71 (41·0)
Arms	56 (14·2)	24 (12·8)	43 (20·3)	42 (24·3)
Hands/wrists	240 (61·1)	134 (71·7)	126 (59·4)	118 (68·2)
Chest	36 (9·2)	20 (10·7)	32 (15·1)	28 (16·2)
Stomach	39 (9·9)	27 (14·4)	43 (20·3)	31 (17·9)
Back	40 (10·2)	21 (11·2)	33 (15·6)	44 (25·4)
Buttocks	25 (6·4)	11 (5·9)	21 (9·9)	28 (16·2)
Popliteal folds	30 (7·6)	11 (5·9)	67 (31·6)	64 (37·0)
Legs	72 (18·3)	21 (11·2)	48 (22·6)	46 (26·6)
Feet/ankles	86 (21·9)	42 (22·5)	61 (28·8)	52 (30·1)
Anogenital region	24 (6·1)	8 (4·3)	20 (9·4)	16 (9·2)

Table 2 Associations between dermatitis on specified skin areas at the time of first appearance and polysensitization based on logistic regression analyses for, respectively, the total population examined and two subpopulations with and without atopic eczema

	Total population (n = 965)		No atopic eczema (n = 580)		With atopic eczema (n = 385)	
Explanatory variables	OR	95% CI	OR	95% CI	OR	95% CI
Scalp	**0·66**	**0·44–0·99**	**0·42**	**0·22–0·82**	0·90	0·50–1·63
Periorbital region	0·85	0·56–1·30	1·38	0·87–2·44	0·55	0·29–1·04
Periauricular region	0·88	0·56–1·37	1·06	0·58–1·91	0·62	0·30–1·29
Perioral region	1·13	0·68–1·90	1·11	0·54–2·30	1·37	0·63–2·96
Remaining part of face	1·20	0·80–1·79	1·10	0·64–1·91	1·27	0·67–2·41
Neck	1·14	0·74–1·74	0·92	0·49–1·71	1·36	0·74–2·53
Shoulders	0·62	0·33–1·18	1·03	0·39–2·75	0·43	0·17–1·05
Armpits	**1·56**	**1·02–2·37**	1·75	0·95–3·20	1·51	0·82–2·77
Cubital folds	1·22	0·80–1·88	1·19	0·56–2·52	1·32	0·74–2·35
Arms	1·10	0·72–1·68	1·09	0·57–2·09	1·27	0·69–2·32
Hands/wrists	**1·58**	**1·18–2·11**	**1·63**	**1·09–2·43**	**1·63**	**1·02–2·61**
Chest	0·99	0·59–1·65	1·13	0·52–2·45	0·94	0·44–2·02
Stomach	0·86	0·54–1·38	1·55	0·81–2·96	**0·40**	**0·19–0·83**
Back	**1·91**	**1·16–3·14**	1·43	0·64–3·19	**2·84**	**1·38–5·84**
Buttocks	1·40	0·78–2·49	0·87	0·33–2·28	1·92	0·86–4·30
Popliteal folds	0·93	0·58–1·48	0·70	0·30–1·64	1·05	0·57–1·94
Legs	0·72	0·48–1·09	**0·50**	**0·27–0·95**	1·05	0·58–1·89
Feet/ankles	0·93	0·67–1·29	1·01	0·65–1·58	0·78	0·47–1·31
Anogenital region	0·81	0·46–1·43	0·77	0·32–1·87	0·88	0·39–1·97

OR, odds ratio; CI, confidence interval. Significant association shown in bold.

ratios (ORs) 1·58 [95% confidence interval (CI) 1·18–2·11], 1·56 (95% CI 1·02–2·37) and 1·91 (95% CI 1·16–3·14), respectively. Dermatitis on the scalp was negatively associated with polysensitization (OR 0·66, 95% CI 0·44–0·99). In the group of patients without AE, dermatitis on the scalp (OR 0·42, 95% CI 0·22–0·82) and on the legs (OR 0·50, 95% CI 0·27–0·95) showed a negative association with polysensitization, and dermatitis on the hands/wrists (OR 1·63, 95% CI

1·09–2·43) a positive association with polysensitization. In the group of patients with AE, dermatitis on the hands/wrists (OR 1·63, 95% CI 1·02–2·61) and on the back (OR 2·84, 95% CI 1·38–5·84) at the time of first appearance was positively associated with polysensitization and dermatitis on the stomach (0·40, 95% CI 0·19–0·83) was negatively associated with polysensitization.

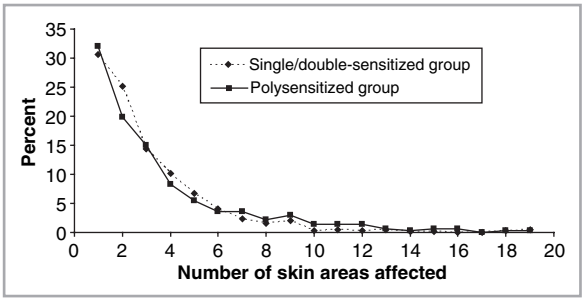

Fig 1. Number of skin areas affected with dermatitis at the time of first appearance compared with number of sensitizations.

Number of skin areas affected at first appearance

The median number of skin areas involved was 2·0 (interquartile range 3·0) for both the single/double-sensitized and the polysensitized groups (Mann–Whitney, $P = 0·299$). Also, when the population was divided according to AE status, no difference between the single/double-sensitized and the polysensitized groups in number of skin areas affected at first appearance was detected (results not shown). Figure 1 illustrates the number of skin areas affected at first appearance compared with the number of sensitizations.

Associations between dermatitis on different skin areas

Nineteen different skin areas could be marked as affected with dermatitis at the time of first appearance with no upper limit on how many skin areas were marked. Figure 2a,b illustrates associations between different skin areas for patients with and without AE. A higher number of associations was found in the AE group. Overall, skin areas on the head were significantly

(a) Atopic eczema

Skin area	Scalp	Periorbital region	Periauricular region	Perioral region	Remaining part of face	Neck	Shoulders	Armpits	Cubital folds	Arms	Hands/wrists	Chest	Stomach	Back	Buttocks	Popliteal folds	Legs	Feet/ankles	Anogenital region
Scalp																			
Periorbital region	3·31																		
Periauricular region	7·03	7·76																	
Perioral region		11·0	5·87																
Remaining part of face	3·47	6·64	5·21	6·53															
Neck	3·77	4·06	5·29	5·62	6·49														
Shoulders					4·74	7·55													
Armpits						4·39													
Cubital folds							3·26												
Arms					2·86	2·88	10·8												
Hands/wrists																			
Chest	3·20	3·20			5·59	4·59	13·8	3·77		7·70									
Stomach				3·52		4·10	8·30	3·38		5·48		12·5							
Back					3·43	3·50	28·1			6·83		6·48	10·4						
Buttocks	3·52					3·60	10·3			5·88		11·3	13·2	10·9					
Popliteal folds								5·02	22·3						3·02				
Legs							10·4			6·19		4·85	6·05	9·43	7·46				
Feet/ankles										3·15	3·00		2·68	3·25	3·57		3·63		
Anogenital region	4·06												4·58		5·71				

(b) No atopic eczema

Skin area	Scalp	Periorbital region	Periauricular region	Perioral region	Remaining part of face	Neck	Shoulders	Armpits	Cubital folds	Arms	Hands/wrists	Chest	Stomach	Back	Buttocks	Popliteal folds	Legs	Feet/ankles	Anogenital region
Scalp																			
Periorbital region	3·40																		
Periauricular region	4·23	4·41																	
Perioral region		5·40	4·23																
Remaining part of face		3·06		4·03															
Neck		3·54		5·19	3·96														
Shoulders						11·3													
Armpits																			
Cubital folds	4·82							5·51											
Arms						4·87	21·4		4·11										
Hands/wrists																			
Chest	3·37					6·27	19·1			5·20									
Stomach						4·33	8·57			3·93		14·1							
Back	3·26					4·93	29·3			6·98		28·4	11·8						
Buttocks							27·6			10·1		10·1	13·5	23·6					
Popliteal folds								5·43	20·4										
Legs							7·58			12·1		3·77	3·39	7·01	4·85				
Feet/ankles																			
Anogenital region																			

Fig 2. Associations between pairs of skin areas affected with dermatitis at the time of first appearance. Numbers represent odds ratios (ORs). The grey squares represent the top quartile of ORs with the highest values.

associated, as well as skin areas on the trunk, and occurrences of dermatitis in the cubital and the popliteal folds were significantly associated with each other. Dermatitis on the hands was for the most part an isolated finding. Further, the feet/ankles and the anogenital region were also isolated locations for dermatitis in individuals without AE.

Discussion

The hands were the most common site of dermatitis in this study for both the groups with and without AE. Sixty-four per cent of all patients (63·4% with AE and 64·5% without AE) reported dermatitis on their hands at the time of first appearance. Frequencies between 25 and 44% have previously been reported in patch test populations, which also included patients with dermatitis with negative patch tests,[2,9–12] and hand dermatitis is also particularly common among adults with AE.[9,10,13] Dermatitis on the hands was associated with polysensitization and was the only area which maintained this association regardless of AE status. This finding is in agreement with another study where the average number of positive patch test reactions per person was significantly higher in individuals with AE with hand dermatitis than without;[14] however, another study reported a significantly lower frequency of hand dermatitis among polysensitized patients than among monosensitized individuals, which could not be confirmed in this study.[2] The skin of the hands is exposed to irritants and allergens. Skin barrier defects caused by dermatitis of any kind and the intense exposure to allergens can easily explain the association to polysensitization. Development of multiple contact allergies may, however, also cause dermatitis on the hands. Hand dermatitis was not associated with dermatitis in other skin areas (except from the feet in the AE group) and can be considered primarily an isolated site of involvement. The time–cause relationship cannot be further elucidated with the cross-sectional design used, but special awareness in patients with hand dermatitis seems justified to prevent development of multiple contact allergies and to document polysensitization as an aetiological factor.

The positive association between polysensitization and dermatitis on the back and the negative association between polysensitization and dermatitis on the stomach was primarily a finding in patients with AE. Patch test reactions can be stronger in patients with multiple allergies due to increased reactivity in the skin,[3] which could lead the study subjects to mark the back as affected at the time of first appearance. The many reactions in themselves could also lead the study subjects to mark the back as affected at the time of first appearance. There is also the possibility of an angry back reaction,[15] i.e. strong positive patch test reactions heighten other patch test responses but the arrangement of the patches on the back seems not to be decisive for development of positive patch tests to neighbouring patch tests.[16–18] Further, exacerbation of preexisting dermatitis may have occurred after patch testing. Patients with AE also often show irritant reactions when patch tested and the combination of many irritant and allergic reactions may more easily be remembered.[19–21] Dermatitis on the stomach could be a remnant of nickel allergy to nickel in trouser buttons. Nickel allergy is predominantly an isolated allergy.[22]

The semiocclusive nature of the armpits and shaving in women may increase skin absorption and lead to an increased sensitization risk. Deodorants are typically the consumer products applied to the armpits which contain multitudes of chemicals. This may explain the association between dermatitis in the armpits and polysensitization. The association was only identified in the total population and not when the population was divided according to AE status, probably because of lesser power.

The majority of patients with scalp dermatitis suffer from endogenous eczema like seborrhoeic dermatitis.[23] This may explain the negative association between polysensitization and scalp dermatitis. Similarly, leg dermatitis was negatively associated with polysensitization but only in patients without AE. Leg dermatitis is more common in patients without AE compared with patients with AE.[9,10] The negative association in this study is surprising as patients with stasis dermatitis are reported to be at risk of polysensitization.[1] Leg dermatitis in this study covers dermatitis anywhere on the leg including both thigh and lower leg and therefore not just stasis dermatitis, which could explain the negative association. Another possibility may be that new topical treatments with low-sensitizing potency developed in recent years may have reduced the previously seen high frequency of polysensitization among patients with stasis dermatitis as seen in one recent study.[24]

Generalized dermatitis (more than two skin areas) was more common in polysensitized compared with monosensitized individuals in one study.[2] This could not be reproduced in this study. The dermatitis was not more widespread at the time of first appearance in polysensitized patients compared with single/double-sensitized patients. Generalization of dermatitis is determined by the exposure pattern which is related to the type of allergy and it is not a result of the number of sensitizations. Even if polysensitized individuals have an increased reactivity as formerly proposed[3] it may result in more severe dermatitis but not necessarily more widespread dermatitis.

The arbitrary division of the skin surface into 19 different regions affects the outcome of these analyses. The face and the trunk were divided into several regions. None of the subdivisions of the face were associated with polysensitization; combining all face-related regions into one did not change the results. On the trunk, the stomach and back were, respectively, negatively and positively associated with polysensitization in patients with AE. Combining the shoulders, chest, stomach, back and buttocks into one region showed no association with polysensitization.

Both cases and controls were recruited from a hospital population minimizing discrepancy in selection factors between the two groups and also decreasing the likelihood of

nonresponse and recall bias. Matching on age and sex was performed so that age and sex were not introduced as confounding factors. Matching on age and time of patch test also minimized differential recall bias.

Patients with ongoing skin problems and more severe dermatitis are more likely to participate and they may remember more correctly. A significantly larger proportion of the polysensitized than the single/double-sensitized group agreed to participate. Mild cases of disease could have been missed and could explain why we did not see any difference in generalization of dermatitis.

Our study subjects were asked to report all the body areas affected with dermatitis at the time of first appearance but some may have reported the cumulative number of skin areas affected throughout the entire period with skin disease which would increase the frequency of involvement of all body areas. Such recall bias is probably nondifferential.

The hands were the only skin area with dermatitis which was associated with polysensitization regardless of AE status. Special awareness in patients with hand dermatitis is recommended to prevent development of multiple contact allergies and to document polysensitization as an aetiological factor.

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