

Retesting with the TRUE Test[®] in a population-based twin cohort with hand eczema – allergies and persistence in an 8-year follow-up study

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Population-based studies on contact allergy with retesting of individuals are infrequently performed. Variable degrees of persistence are reported when individuals with contact allergy are retested with years in between. The patch test results of 270 individuals tested in 2005–2006 are presented and the pattern and frequency of sensitization discussed. Persistence when compared with patch test results from 1997–1998 is reported. 270 twin individuals with and without hand eczema underwent patch testing with the TRUE Test[®] (Mekos Laboratories AS, Hilleroed, Denmark) in 1997–1998 and again in 2005–2006 as part of a larger study. In 2005–2006, a total of 74 (27.4%) of the 270 individuals had at least 1 positive patch test and 20 (7.4%) of the 270 had 2. The frequency in men and women was 9/90 (10%) and 65/180 (36.1%), respectively. The frequency of contact allergy in individuals with and without hand eczema was 59/185 (31.3%) and 15/85 (17.6%), respectively. The most prevalent contact allergies were to nickel, thiomersal, and fragrance mix I. All together, 74% of the positive reactions were reproduced. The frequency of contact allergy in this population-based cohort with hand eczema was comparable with previous reports. Persistence of contact allergy after many years was confirmed.

Key words: contact allergy; hand eczema; patch testing; persistence; retesting. © Blackwell Munksgaard, 2007.

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A number of screening studies on contact allergy in the general population has been performed, and to our knowledge, only 1 reports retesting. Nielsen et al. (1) patch tested 290 and 469 individuals randomly chosen from the Danish population in 1990 and 1998, respectively. In addition, in another study, 1146 Danish school children aged 12–16 years were patch tested (2). In a Norwegian study, 1236 randomly selected adults underwent patch testing (3). Meding et al. (4) patch tested a population-based cohort of 1081 individuals with hand eczema. Often hospital-based patch tests results from patients with eczema are reported (5–7). The proportion of sensitized individuals and the nature of the involved allergens vary depending on the test population.

Contact allergy is diagnosed by means of a patch test, using either the chamber system or a ready-to-use system (the TRUE Test[®]; Mekos Laboratories AS, Hilleroed, Denmark). Once an individual is sensitized, the allergy is generally believed to persist lifelong. However, when retest-

ing individuals with contact allergy after a variable time period (3–12 years), a persistence of positive reactions between 66% and 86% is reported (8–10), indicating that the recommended patch test concentration is no longer capable of eliciting a positive patch test reaction.

In the present study, results from patch testing of 270 twin individuals with and without hand eczema in 1997–1998 and again in 2005–2006 are presented. The frequency of sensitization, the pattern of sensitization, and the persistence of positive reactions are discussed.

Materials and Methods

Study population

In 1997–1998, a total of 1076 adult twin individuals participated in a clinical examination, interview, and patch testing. The twin individuals were ascertained from a population-based twin cohort of 5610 same-sex twins participating in a questionnaire survey on hand eczema in 1996 (11, 12). If both

twins in a twin pair had returned the questionnaire and at least one of them had reported symptoms of hand eczema, they were invited to participate. In addition, both twin individuals in a twin pair had to live within 60 km from Copenhagen.

In 2005–2006, twin individuals with self-reported hand eczema or with a co-twin with self-reported hand eczema in the questionnaire survey of 1996 were selected to participate in a new clinical examination and patch testing (659 twin individuals). Addresses were obtained from the Danish Civil Registration System on 605 twin individuals. The remaining twin individuals had a protected address, had emigrated, or had died. Participants were enrolled after informed consent was obtained, in compliance with the principles of the Helsinki Declaration.

Patch testing

All participants received the ready-to-use TRUE Test[®] system (Mekos Laboratories AS) by mail and were asked to keep the test material refrigerated. They were given instruction on how to place the patches and how to mark the location with a pen in a written instruction enclosed with the patch test. In 2005–2006, all participants also received a phone call and were given oral information about the test procedure and possible adverse reactions. Testing was performed throughout the year in 1997–1998. In 2005–2006, no testing took place during the summer months (July and August). 3 days in advance of the scheduled examination, patches were placed on the upper back by the participants. The patches were removed again after 2 days by the participants. Reading of the patches was performed on D3 according to the International Contact Dermatitis Research Group guidelines (13). A + reaction was defined as homogeneous redness and infiltration in the test area, possibly with additional papules. All readings were performed by Lars Erik Bryld (1997–1998) (14) or A. L. (2005–2006) in collaboration with 2 experienced nurses. Only + to +++ reactions were considered positive. In 2005–2006, 6 individuals removed an allergen from the test panel (nickel) because of a previous strong patch test reaction. If the allergen had not been removed, a positive reaction would be anticipated, and in the calculation on persistence, they are counted as persistent reactions.

Statistical analyses

Data management and descriptive statistical analyses were performed in SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). The chi-squared test was used in the drop-out analysis. *P* values are 2 sided and a cut-off level for statistical significance of 5% was used.

Results

A total of 274 twin individuals (183 women and 91 men) with a mean age of 42 years (range 29–55 years; SD = 6.5) volunteered to participate in the patch testing. 4 individuals were not patch tested because of breastfeeding or systemic immunosuppressive therapy, resulting in a participation rate of 41%. The group comprised 86 twin pairs and 102 single-twin individuals (106 monozygotic twin individuals, 153 dizygotic twin individuals, and 15 with unknown zygosity). The mean follow-up period was 8.6 years (range 7.3–9.4 years). A subgroup of 188 had self-reported hand eczema. This group has been described in detail elsewhere (15).

Contact sensitivity

In 1997–1998, a total of 65 (23.7%) of the 274 individuals had 1 or more allergies. 2 allergies were detected in 16 (5.8%) of the 274 individuals, and 3 (1.1%) of the 274 individuals had 3 positive patch tests. At the second patch testing, 74 (27.4%) of the 270 individuals had at least 1 positive patch test, and 20 (7.4%) of the 270 had 2 positive patch tests. The frequency of a positive patch test was 9 (10%) of 90 and 65 (36.1%) of 180 in men and women, respectively. None had more than 2 positive reactions at the second patch testing. The frequency of contact sensitivity in individuals with and without hand eczema was 59 (31.3%) of 185 and 15 (17.6%) of 85, respectively. No individuals returned with new reactions emerging after the reading on D3.

Allergens

Table 1 shows a list of previous and present positive reactions as well as number of lost and new allergies. In addition, the distribution of contact sensitivity in individuals with and without hand eczema is displayed. Nickel allergy was the most prevalent allergy, followed by contact sensitivity to thiomersal and fragrance mix. 14 incident cases with nickel allergy were recorded, all but 1 were in women. 7 of the incident cases had hand eczema. There were no positive reactions to wool alcohols, neomycin sulfate, caine mix, quinoline mix, quaternium 15, and mercaptobenzothiazole.

Persistence

Overall, 64 (74%) of 87 positive reactions in 1997–1998 were reproduced at the second patch testing in 2005–2006. The highest persistence was found for +++ reactions [100% (10 of 10)], whereas 69% (29 of 42) and 71% (25 of 35) of the ++ and + reactions persisted. Allergen-specific

Table 1. Number of positive reactions (+, ++, or +++) to allergens in the TRUE Test^{®a}

Allergen	Previous study, <i>n</i> = 274, total	Present study, <i>n</i> = 270					Comparison: previous and present studies		
		Women, <i>n</i> = 180	Men, <i>n</i> = 90	Total	Hand eczema, <i>n</i> = 185	No hand eczema, <i>n</i> = 85	New	Lost	Persisted
Nickel sulfate	46 (16.8)	48 (23.3)	4 (4.4)	52 (19.3)	40 (21.6)	12 (14.1)	14	8	38 (83)
Potassium dichromate	1 (0.4)	0	0	0	0	0	0	1	0 (0)
Fragrance mix	7 (2.6)	7 (3.9)	0	7 (2.6)	6 (3.2)	1 (1.2)	1	1	6 (86)
Colophony	5 (1.8)	2 (1.1)	2 (2.2)	4 (1.5)	4 (2.1)	0	0	1	4 (80)
Epoxy resin	6 (2.2)	3 (1.6)	0	3 (1.1)	1 (0.5)	2 (2.3)	0	3	3 (50)
Balsam of Peru	0	1 (0.6)	0	1 (0.4)	1 (0.5)	0	1	—	—
ED	1 (0.4)	0	0	0	0	0	0	1	0
Cobalt dichloride	4 (1.5)	3 (1.7)	0	3 (1.1)	3 (1.6)	0	0	1	3 (75)
<i>p</i> - <i>t</i> -BFR	5 (1.8)	2 (1.1)	2 (2.2)	4 (1.5)	3 (1.6)	1 (1.2)	1	2	3 (60)
Paraben mix	0	1 (0.6)	0	1 (0.4)	0	1 (1.2)	1	—	—
Cl+ Me- Isothiazolinone	2 (0.7)	1 (0.6)	2 (2.2)	3 (1.1)	3 (1.6)	0	1	0	2 (100)
PPD	0	1 (0.6)	1 (1.1)	2 (0.7)	2 (1.1)	0	2	—	—
Formaldehyde	0	1 (0.6)	1 (1.1)	2 (0.7)	2 (1.1)	0	2	—	—
Mercapto mix	1 (0.4)	0	0	0	0	0	0	1	0 (0)
Thiomersal	6 (2.2)	10 (5.6)	0	10 (3.7)	8 (4.3)	2 (2.3)	6	2	4 (67)
Thiuram mix	3 (1.1)	2 (1.1)	0	2 (0.7)	2 (1.1)	0	1	2	1 (34)
Total	87	82	12	94	75	19	30	23	64 (74)

ED, ethylenediamine dihydrochloride; Cl+ Me- Isothiazolinone, 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one; PPD, *p*-phenylenediamine; *p*-*t*-BFR, *p*-*tert*-butylphenol formaldehyde resin.

^aPercentages are given in parentheses.

persistence rates are displayed in Table 1. In 1997–1998, 15 doubtful reactions were recorded; none of them became positive in 2005–2006. Of 38 doubtful reactions determined in 2005–2006, 2 had been positive in 1997–1998.

Drop-out analysis

Drop-out analysis of the 274 twin individuals participating in the present study compared with twin individuals where one or both had self-reported hand eczema in 1997–1998 (659) showed no statistically significant difference regarding sex, zygosity, hand eczema status in 1997–1998, co-twins hand eczema status in 1997–1998, or patch test status (data not shown). Age was the only statistically significant factor influencing willingness to participate. The twin individuals were subdivided into 3 groups, and 35%, 41%, and 50% participated from the youngest, middle, and oldest age groups, respectively ($P = 0.009$).

Discussion

In this population-based study of 270 individuals, we found a frequency of positive patch test reactions in 31.3% and 17.6% individuals with and without hand eczema, respectively. These frequencies are comparable with previous reports. Meding et al. (16) found that 32% of individuals with hand eczema recruited from the background popu-

lation had a positive patch test. Nielsen et al. (1) determined the frequency of contact allergy in the general population in 1990 and 1998 and found estimates on 15.9% and 18.6%, respectively. Recently, in a population-based Norwegian study, at least 1 positive patch test was found in 26.3% of individuals (3). In another recent population-based study on school children, the prevalence of contact allergy was 15.2% (2).

As reading in the present study was performed only after 3 D, late reactions may be missed and the frequency of positive reactions may be underestimated. It has been shown that between 3% and 8.2% of reactions become positive on D6 or D7 (17, 18). In the studies mentioned above on cohorts from the general population, reading was performed on D2 or D3, thus a comparison seems justified. As no individuals returned with late reactions appearing after the reading was performed, we believe that there was no active sensitization during the test procedure, indicating that the ready-to-use patch test system is an acceptable diagnostic procedure.

The present study was part of a larger twin study, with the main focus on genetic risk factors for hand eczema, taking advantage of the methodological possibilities in a twin study design (19, 20). The twin design was not used in the present study. Genetic susceptibility to contact allergy cannot be entirely excluded, but allergenic exposure seems more important for the risk of contact allergy (21). Should genetic susceptibility to contact

allergy significantly influence the risk of contact allergy, then a higher concordance rate for contact allergy between monozygotic twin individuals than between dizygotic twin individuals would be anticipated; however, this would not influence the frequency estimates. Thus, the frequency estimates obtained in this study can be compared with those obtained in a non-twin population. No studies indicate that twin individuals should be more susceptible to contact allergy in general; thus, the results from this study can be extrapolated to a non-twin population.

The higher frequency of positive reactions in individuals with previous or present hand eczema was primarily a result of an excess frequency of positive reactions to nickel, thiomersal, and fragrance mix I. In addition, sensitization to preservatives (CI+ Me- Isothiazolinone and formaldehyde), colophony, cobalt, thiuram mix, *p*-phenylenediamine, and Balsam of Peru was only found in individuals with hand eczema. An association between nickel allergy and hand eczema has previously been reported (22, 23); however, in a recent study, this association could not be confirmed (24). 14 new cases of nickel sensitization were identified. As the Danish regulation on nickel release from metal objects in close contact with the skin came into force in 1991 and in the EU in 2000, the reported prevalence of nickel allergy has decreased (25). However, as shown, sensitization still occurs, and one may speculate whether sensitization is because of occupational exposure, violation of the nickel regulative, or exposure to objects with a higher nickel content from outside Denmark or EU. Sensitization to thiomersal may occur through vaccination, contact with eye drops, contact lens solutions, or cutting oils and fuels, where thiomersal is added as a preservative. However, the allergy is generally believed to be without clinical significance, and thiomersal is not included in the European Standard Series (26). The frequency of sensitization to thiomersal found in this study is relatively high compared with previous studies (2, 3). Fragrance mix I, balsam of Peru, and colophony are all indicators of contact allergy to fragrances. A possible association between hand eczema and fragrance allergy has been proposed, but this has not been confirmed (27). A new fragrance mix (fragrance mix II) has been developed, and it has been shown that about one-third of the patients with a positive test reaction to fragrance mix II are negative to fragrance mix I; thus, some cases of fragrance contact allergy may have been missed in this study (28). Sensitization to cobalt usually occurs in relation to contact allergy to nickel or chromate because of simultaneous exposure (29). Two of the three cases in this study with cobalt sensitization

had concomitant nickel allergy. Sensitization to the rubber chemicals included in the thiuram mix often occur through contact with rubber gloves; thus, the presence of thiuram allergy in individuals with previous or present hand eczema is not surprising.

Because of the limited number of tested individuals, statistical analyses on possible associations between contact allergy and hand eczema or between sensitization to specific allergens and hand eczema were not attempted. In addition, judgement on the relevance of patch test reactions was not attempted as many individuals had only historic symptoms. The higher frequency of contact allergy in women is well-known and is primarily a result of a high frequency of nickel and fragrance allergy in women (1).

After a mean follow-up period of 8.6 years, 74% of the previously positive reactions persisted. The number of persistent reactions clearly depends on the reproducibility of the patch test procedure. The degree of reproducibility depends on methodological variation, seasonal variation, potential ultraviolet exposure, and active eczema. In addition, persistence relates to (an unexplained) individual fluctuation in reactivity and possible decreased immunological reactivity over time (30). Simultaneous patch testing with 2 identical TRUE Test[®] panels has proven high rates of reproducibility (95%), and higher reproducibility is reported when using the ready-to-use test system than the chamber system (31, 32). Reading of the patch test reactions at the first and second examination was performed by 2 different persons; however, both were educated in the same department and experienced with the patch test procedure. Notably, the degree of persistence of + and ++ reactions was equal. This probably reflects the conservative interpretation of the guidelines from the International Contact Dermatitis Research Group (a + reaction requires at least homogeneous redness and infiltration in the test area), which is used in the department. The main cause of methodological variation in the patch test procedure in this study is probably the fact that participants applied and removed the patches themselves. Thus, errors and inconsistencies in the test procedure cannot be excluded, even though the participants were carefully instructed.

With a participation rate of 41%, bias owing to drop-out becomes a concern. Drop-out analysis showed no statistically significant difference with regards to sex, hand eczema, or patch test status in 1997–1998. However, an increased tendency for individuals with recurrent or more severe symptoms of allergic contact dermatitis to attend cannot be excluded, and if so, this may increase the possibility of a persistent positive patch test.

In conclusion, the frequencies of contact allergy found in this study were comparable with those in previous reports; 31.3% and 17.6% in individuals with and without hand eczema, respectively. The most frequent involved allergens were nickel, thiomersal, and fragrance mix I. The persistence of contact sensitivity even after many years was confirmed in this study as 74% of previous positive reactions remained positive. Allergen avoidance should be encouraged in patients with a positive patch test.

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