FACULTY OF HEALTH SCIENCES
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PhD Thesis

Epidemiological and clinical studies on hand eczema in a population-based twin sample

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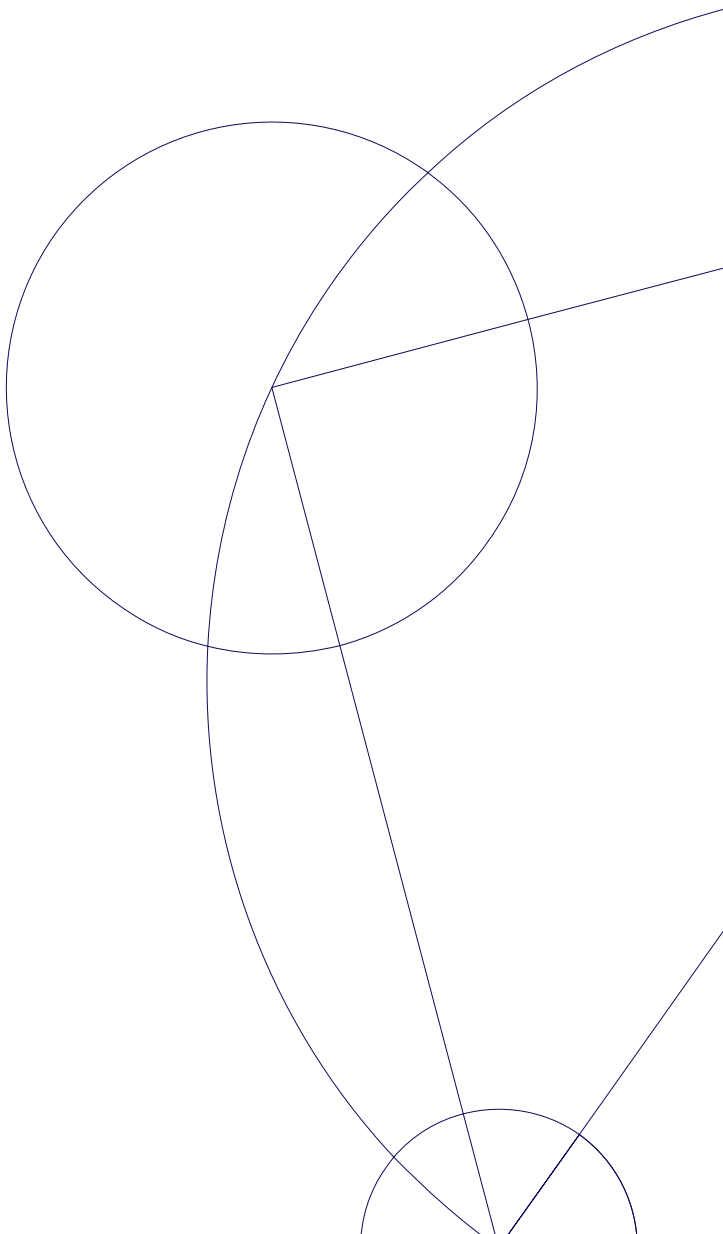

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This PhD thesis is based on the following manuscripts:

Study Part 1

I. Lerbaek A, Kyvik KO, Mortensen J, Bryld LE, Menné T, Agner T. Heritability of Hand Eczema is Not Explained by Comorbidity with Atopic Dermatitis. *Journal of Investigative Dermatology 2007; 127: 1632-40.*

II. Lerbaek A, Kyvik KO, Ravn H, Menné T, Agner T. Incidence of hand eczema in a population-based twin cohort: genetic and environmental risk factors. *British Journal of Dermatology 2007; 552-7.*

Study Part 2

III. Lerbaek A, Kyvik KO, Ravn H, Menné T, Agner T. Clinical characteristics and consequences of hand eczema – an eight-year follow-up study of a population-based twin cohort. Manuscript submitted to *Contact Dermatitis 2007.*

IV. Lerbaek A, Kyvik KO, Menné T, Agner T. Retesting with the TRUE Test® in a population-based twin cohort – allergies and persistence in an eight-year follow-up study. *Contact Dermatitis 2007; 57: 248-52.*

V. Lerbaek A, Agner T, Kyvik KO, Bisgaard H, Palmer CNA, Menné T. Filaggrin null alleles are not associated with hand eczema or contact allergy. Accepted for publication in *British Journal of Dermatology 2007*.

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Anne Lerbæk Sørensen

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TABLE OF CONTENTS

1 INTRODUCTION

Years of clinical and epidemiological research in hand eczema has elucidated important risk factors for the disease and provided insight into the complex aetiology. However, hand eczema is still a very common and often chronic disease with considerable societal expense and personal cost, of both an economical and social nature.

Clustering of disease in families is well known from clinical practice and can be due to shared environment or shared genes within the family. This thesis builds on a genetic-epidemiological study from 1996–99 on hand eczema in twins[1]. The present study focused on identifying genetic and environmental risk factors for hand eczema; to determine the incidence rate of the disease, and to describe its typical clinical characteristics and prognosis.

2 BACKGROUND

2.1 Defining hand eczema

Hand eczema is a heterogeneous disease and no real "gold standard" for its clinical diagnosis exists. In clinical practice the diagnosis is based on characteristic symptoms and exclusion of other diagnoses (typically dermatophytoses and psoriasis). An aetiological subclassification relies upon a thourough history (atopic disposition, domestic and occupational exposures), patch testing, and clinical manifestations; clinical manifestations alone cannot be relied on for subclassification[2-4]. Hand eczema is usually subclassified into hand eczema due to allergic contact dermatitis, irritant contact dermatitis, atopic dermatitis or combinations of the three[4-7]. In addition vesicular hand eczema, hyperkeratotic hand eczema and hand eczema due to or aggravated by contact urticaria comprise minor groups[4-7] and some authors also specify a group with nummular eczema[5;7]. Skin biopsy is not helpful to distinguish the different subtypes[4;8;9].

In larger population-based epidemiological studies clinical examination of participants is usually impossible for practical and economic reasons. A questionnaire-based approach provides a pragmatic alternative way to evaluate occurrence of hand eczema in larger cohorts. Two different questionnaire-based strategies have been employed: a diagnosis based on a self-report of symptoms and a diagnosis based on a self-report of hand eczema. The diagnosis based on a self-report of symptoms has shown a high sensitivity, but a low specificity[10]. The self-report of hand eczema has a low sensitivity and high specificity[10-12].

2.2 Epidemiology of hand eczema

In population-based studies on adults the point prevalence of hand eczema is between 3.3% and 5.4%[13-15]. In school children aged 12–16 years and 16–18 years a point prevalence of 3.2% and 4.1–5.6%, respectively, has been estimated[16;17]. In certain occupational groups the point prevalence is

increased, hence the point prevalence in rubber workers, workers in the printing industry and in office workers is reportedly 6.9%, 11.0% and 7.2%–10.0%, respectively[18-20].

Estimates on the 1-year prevalence of hand eczema in population-based studies varies between 8% and 11.8% in adults[13;21-23] and between 7.3% and 10.5% in school children[16;17]. Increased 1-year prevalence has been reported in car mechanics and dentists (15%)[11].

Reports on the lifetime prevalence of hand eczema are less frequent. Bryld et al found an estimate of 17.0%[14] and Meding et al reported a very similar estimate of 17.4%[24]. In another Swedish study the reported lifetime prevalence varied between 5.9% and 12.8% in different age groups[25].

The number of studies on incidence is limited. Retrospective studies on the incidence rate of hand eczema in the general population have found estimates ranging from 4.4 to 7.9 cases per 1000 person-years[5;24;26]. Very high estimates have been found in high-risk groups such as nurse and hair dressing apprentices (145–328 cases per 1000 person-years)[27;28]. Recently, Lind et al reported an incidence rate of 23.8 cases per 1000 person-years in hair dressers[29]. In office workers an incidence rate of 41 cases per 1000 person-years has been reported[20].

Hand eczema due to irritant contact dermatitis is the most common diagnosis, both in population-based samples and when looking at occupational hand eczema, followed by allergic contact dermatitis and atopic dermatitis on the hands[5-7;30-32].

2.3 Risk factors for hand eczema

Risk factors for hand eczema comprise both exogenous and endogenous factors. A history of atopic dermatitis has long been recognised as one of the main risk factors for hand eczema[16;21;33-36]. In some studies[26;33;35], but not all[37;38], respiratory atopy is also a risk factor. Contact allergy may elicit allergic contact dermatitis on the hands and is an important but less prominent risk factor for hand eczema than atopic dermatitis[21;39-41].

Wet work is a third important risk factor[21;35;42]. High-risk occupations with increased risk of hand eczema due to exposure to wet work and/or allergens include bakers, hairdressers, dental surgery assistants, kitchen workers/cooks, butchers, health-care workers, cleaners, doctors/dentists/veterinarians and laboratory technicians[6].

It is repeatedly shown that hand eczema is approximately twice as common in women as in men in young age groups[7;23;24;43]. This sex-difference is thought to be related to difference in skin exposure and not to differences in skin susceptibility to irritants and allergens between the sexes[44]. No sex-difference in skin reactivity to irritants has been found[45]. Young age is associated with a high prevalence and incidence rate[13;23;24]. Having children below the age of four and not having a dish washing machine at home are significant risk factors[42].

Whether tobacco smoking is associated with risk of hand eczema is uncertain. A few studies have evaluated the subject and the results are conflicting[25;36;46;47]. No reports on alcohol consumption as a potential risk factor for hand eczema have been found.

2.4 Genetic risk factors for hand eczema

In the previous twin study on hand eczema it was demonstrated that genetic factors influence the risk of developing hand eczema. The heritability estimate was 0.65 (95% confidence interval 0.33–0.93)[14]. Further analysis of the data indicated that the heritability was not due to atopic dermatitis or contact allergy; however, this was statistically insignificant[41]. It is well known that genetic factors significantly influence the risk of atopic dermatitis[48]. Thus, the genetic effects influencing the risk of hand eczema could partly be explained by comorbidity with atopic dermatitis.

Whether genetic factors influence the risk of acquiring contact allergy has been a subject of research for many years. In guinea pig studies breeding of low and high responder animals to sensitization with DNCB has been demonstrated[49]. Further, different sensitization potentials to different allergens in different inbred guinea pig strains have been reported[50]. A human sensitization study demonstrated increased susceptibility in children of sensitized parents[51]. However, in twin studies a greater resemblance between monozygotic (MZ) twins than between dizygotic (DZ) twins with respect to DNCB sensitization, positive patch test reactions to 23 allergens or patch test positivity to thiomersal could *not* be found[52;53]. Menné and Holm found a significantly increased concordance rate of a history of nickel allergy in monozygotic twins compared to dizygotic twins[54]. Bryld et al concluded that nickel allergy is mainly caused by environmental and to a lesser degree by genetic factors[55]. Several studies explored a possible association between HLA-markers and contact allergy, but none has been successfully replicated and in most cases no association was found[56-64]. Recently, association between two cytokine gene polymorphisms and contact allergy[65;66] and between a polymorphism of gluthatione S-transferase M1 and contact allergy to thiomersal was reported[67]. Also, association between genetic polymorphisms of N-acetyltransferases and contact allergy to para-substituted arylamine compounds (i.e. p-Phenylenediamine, benzocaine etc) has been demonstrated[68-70].

Another approach has been to focus on polysensitized individuals. Moss et al found that individuals with multiple contact allergies were more readily sensitized with DNCB than individuals without multiple contact allergies[71]. Brasch et al showed that strong positive reactions to nickel or fragrance mix were associated with an increased risk of further positive reactions to structurally unrelated allergens. This was interpreted as an indication of a general disposition to contact allergy in individuals with strong patch test reactions[72]. Recently, an association between polysensitization and sensitization to weak contact allergens (paraben mix) was reported[73].

Much less is known about individual genetic susceptibility to irritant contact dermatitis other than atopic dermatitis. Holst and Möller compared skin reactivity in MZ and DZ twin pairs. They found a significantly higher concordance rate on patch test reactions in MZ twins when testing with sapo

kalinus, but not with benzalkonium chloride and sodium lauryl sulphate[74]. Also, irritant contact dermatitis has been related to a TNF-α gene polymorphism[75]; however, the same marker has also been shown to be associated with contact allergy[66].

A strong association between two loss-of-function mutations (null alleles), R501X and 2282del4 in the gene encoding filaggrin, and atopic dermatitis has recently been established [76]. Filaggrin is an essential protein in stratum corneum, important for the skin barrier function. The variant alleles result in a decreased amount or complete loss of filaggrin products[77]. Any possible association between the filaggrin variant alleles and hand eczema or contact allergy is unexplored.

2.5 Prognosis of hand eczema

A tendency for prolonged symptoms in many hand eczema patients is well-documented[78-81]. In a 15-year follow-up study on a population-based cohort with hand eczema, 28% reported symptoms ≥ ½ time, 38% reported symptoms < ½ time and 34% had no symptoms in the follow-up period[82]. Young age at onset, atopic dermatitis, widespread eczema, polymorphism with respect to visible signs of hand eczema, and having seen a doctor because of hand eczema have been shown to be negative prognostic factors[83;84]. The potential occupational consequences of hand eczema are considerable and include sick leave and job change[78;79;82;85]. Factors shown to increase the risk of sick leave include hand eczema due to allergic contact dermatitis, atopic dermatitis, greater age and lower socioeconomic status[86-88].

2.6 The use of twins in medical research

Sir Francis Galton (1822-1911) was an English scientist and distant relative to Charles Darwin. In 1875 in "The history of twins" he was the first to describe the idea of studying twins as a way to discriminate between the effect of inherited (genetic) qualities and the effect of environmental influence[89]. The later developments in statistical methods and the establishment of comprehensive twin registries paved the way for systematic and extensive twin studies.

MZ twins arise from a single fertilized ovum and the classical twin study relies on the assumption that MZ twins have identical segregating genes. Any difference (discordance) for a disease in a MZ twin pair is thus attributed to environmental influence. DZ twins share on average 50% of their genes as do ordinary siblings and any difference is therefore due to a combination of genetic and environmental factors. Any substantial influence from genetic factors on disease liability can be detected as a greater phenotypic similarity between MZ twin individuals than between DZ twin individuals[90]. The classical twin study further relies on two important assumptions. First, MZ and DZ twins must share the environment to the same degree. If this is not the case, increased resemblance between MZ twins can also be due to a greater degree of environmental similarity. Second, extrapolating the results to the general (non-twin) population requires that the twin population is representative of the general population.

3 AIMS OF THE STUDY

The aims of this thesis were to:

Part 1: The questionnaire study

- Investigate the relative importance of genetic and environmental risk factors for hand eczema independent of atopic dermatitis
- Investigate whether genetic risk factors influence the frequency of eruptions of hand eczema or the age at onset
- Determine the incidence rate of hand eczema and factors influencing this rate

Part 2: The clinical study

- Investigate the clinical characteristics, occupational and medical consequences of having hand eczema and factors influencing persistence of hand eczema
- Assess the outcome of a second patch testing of twin individuals patch tested eight years apart with the TRUE Test®
- Investigate any association between the filaggrin null alleles, R501X and 2282del4, and hand eczema or contact allergy

4 MATERIALS AND METHODS

The work described in this thesis is the result of two separate but related projects: a questionnaire study (part 1) on a large cohort comprising 4128 twin individuals and a clinical study (part 2) comprising 274 twin individuals.

4.1 Part 1: The questionnaire study

4.1.1 Study population and design

In 1996 a questionnaire was sent to 6666 same-sex twins ascertained from the Danish Twin Registry. The twins were born between 1953 and 1976 and lived on Zealand and neighbouring islands. The cohort comprised MZ and DZ twin pairs, a few triplets and quadruplets and a minor group with unknown zygosity (UZ); 5610 twin individuals were available for analysis. The results from this study have been reported[14].

In January 2005 a second questionnaire was sent to eligible individuals together with a stamped self-addressed return envelope. Non-respondents received one reminder after one month. Updated addresses were requested from the Danish Civil Registration System and were obtained for 5048 individuals. The remaining individuals had protected or unknown addresses (409 individuals), had emigrated (96 individuals) or died (57 individuals).

4.1.2 Questionnaires

The 1996 questionnaire included 10 questions on self-reported hand eczema, doctor-diagnosed hand eczema and symptoms of hand eczema[14].

Only twin individuals *without* self-reported hand eczema in 1996, where asked about self-reported hand eczema in the 2005 questionnaire. In total 4223 questionnaires were sent to twin individuals without previously self-reported hand eczema and 825 questionnaires were sent to twin individuals with previously self-reported hand eczema.

Fourteen twin individuals reporting hand eczema in 1996 made a marginal note in the questionnaire denying previous hand eczema and in the data analyses they were merged with the group without self-reported hand eczema.

The questionnaires also included questions on atopic dermatitis (UK Working Party's Diagnostic Criteria, question-only version[91-94]) and questions on positive patch tests to nickel or other contact allergens (preservatives, perfume, rubber, plants, chromate or other). A question from The Nordic Occupational Skin Questionnaire (NOSQ-2002) (question number D4) was used to assess the frequency of eruptions[95]. A question on job title was used to assess skin exposure indirectly, whereas questions on hours of wet work per day, hours with glove use per day and number of hand washings per day were used as a more direct estimate of skin exposure (questions adapted from the NOSQ-2002; question numbers E1, E2, E5 and E8)[95]. Coherence and comprehension of the questions were

evaluated in a pilot test with 15 hand eczema outpatients from the Department of Dermatology and 15 individuals without hand eczema recruited among colleagues and friends. Questions were considered relevant and easy to understand.

4.1.3 Definitions

A diagnosis of s*elf-reported hand eczema* was defined as "yes" to one of the questions "Have you ever had hand eczema?" or "Has a doctor ever told you that you have hand eczema?". Concerning *frequency of eruptions* respondents were divided into four groups, having had hand eczema (1) never, (2) only once, (3) more than once and (4) (nearly) all the time. This division was inspired by the response options recommended in the NOSQ-2002. The calendar year of first episode of hand eczema was subtracted from the birth year to obtain the *age at onset*. *Atopic dermatitis* was defined using the UK Working Party's Diagnostic Criteria, question-only version[94]. A positive answer to a question on positive patch tests was considered indicative of *contact allergy*. *High-risk occupation* was defined as employment in one of 9 occupations, previously identified as high-risk occupations for hand eczema (bakers, hairdressers, dental surgery assistants, kitchen workers/cooks, butchers, health care workers, cleaners, doctors/dentists/veterinarians and laboratory technicians)[6]. *Wet work* was defined as skin exposed to liquids more than two hours per day, or use of occlusive gloves more than two hours per day, or very frequent hand washing (>20 times/day)[96]. Based on *smoking* history individuals were primarily stratified into three separate categories: (1) never smokers, (2) current smokers and (3) ex-smokers; and secondarily on the basis of pack-years (1 pack-year = 15 cigarettes per day for 1 year) into the following categories: (1) 0 pack-years, (2) ≤ 15 pack-years and (3) > 15 pack-years. Based on open questions on mean weekly intake of beer (bottles), wine (glasses) and spirits (units), individuals were divided into three categories depending on *alcohol* intake: (1) never, (2) ≤ 21 drinks/week and (3) > 21 drinks/week. One drink was defined as one bottle of beer, one glass of wine or one unit of spirits.

4.1.4 Data entering

The questionnaires were scanned by a professional company specialized in scanning of questionnaires (UNI-C). The resulting data material was checked for errors by (1) manual data check of 50 randomly chosen questionnaires, (2) frequency tables were made for all variables to check for outlying values and (3) control for internal consistency by extensive cross tabulations. In the case of errors or inconsistencies values were checked with the original questionnaires.

The frequency of missing values varied widely with the question. For most questions the frequency of missing values was less than 4% (for the majority around 1%); however, a few questions were apparently difficult to answer. Thus, almost 9% of the twin individuals with self-reported hand eczema did not answer the question on year of onset and about 5% did not state frequency of eruptions. In the question on job titles individuals with a known high-risk occupation could put a cross in a pre-specified category, whereas the remaining individuals were asked to handwrite their job title by hand. However, information on this variable was missing in 22.1%.

4.1.5 Statistical analyses

4.1.5.1 Manuscript I

Data on all twin individuals (MZ, DZ, UZ, triplets and quadruplets; single twin individuals and complete twin pairs) were used in the descriptive analyses. Data management, descriptive statistical analyses, and the chi squared (χ^2) test (drop-out analysis), were done in SPSS version 13.0[97].

Twin similarity was assessed using casewise concordances, conditional probabilities and correlation coefficients, stratified by zygosity and sex. This was followed by quantitative genetic modelling to estimate the relative importance of genetic and environmental factors. Only data on complete MZ and DZ twin pairs were used in the twin analyses.

Casewise concordances were calculated for self-reported hand eczema, high-risk occupations and wet work (the latter two in order to investigate the correctness of the equal environment assumption) using Stata Statistical Software[98]. Similarity in frequency of eruptions was assessed with conditional probabilities for variables with more than two outcomes using Maple version 10[99]. 95% confidence intervals were calculated using bootstrap methods[100]. Equality between MZ and DZ concordances was tested in a one-sided test.

Tetrachoric correlation coefficients or polychoric correlation coefficients were calculated for self-reported hand eczema and frequency of eruptions, respectively, to express the correlation in liability within twin pairs. Age and atopic dermatitis were included in the models assuming a linear effect on the thresholds by the covariates. The difference in tetra- and polychoric correlation coefficients between MZ and DZ twins was assessed with a likelihood ratio test. Correlations in age at onset were assessed with Pearson correlation coefficients.

In quantitative genetic modelling, the total phenotypic variance (V_P) is decomposed into four variance components: additive genetic variance (the influence of alleles at several loci acting in an additive manner -V_A), non-additive genetic variance (the presence of dominance, which is non-additive interaction of alleles at the same locus or the presence of epistasis, which is interaction between alleles at different loci - V_D), common environmental variance (environmental influence shared by members of a twin pair - V_C), and unique environmental variance (environmental influence unique to the individual - V_E)[101]. MZ twin pairs are perfectly correlated for genetic variance (V_A+V_D), whereas additive variance is correlated 0.5 and dominant genetic variance 0.25 across DZ twin pairs. Common environment by definition is perfectly correlated between both MZ and DZ twin pairs. Unique environment is uncorrelated in both zygosity groups. Based on this, the equations for the expected variances and twin-covariances are as follows:

$V_P = V_A + V_D + V_C + V_E$

$COV_{MZ} = V_A + V_D + V_C$

$COV_{DZ} = 0.5\ V_A + 0.25\ V_D + V_C$

where COV_{MZ} is the covariance within MZ twin pairs and COV_{DZ} is the covariance within DZ twin pairs.

The modelling procedure tests these expected variance-covariance matrices against the observed data pattern, and the aim is to explain the pattern with the use of as few parameters as possible. First, full models (ADE and ACE) were examined, stratified by sex, and secondly a model assuming equal variance components for both sexes was attempted. Equal variance components across sex were confirmed and then nested submodels (AE, DE and E and AE, CE and E, respectively) were computed on a dataset comprising both sexes. Same procedure was followed with the covariates, atopic dermatitis and age, included in the model. It was not possible to include further covariates in the model. Instead, in new calculations on self-reported hand eczema all twin individuals reporting a positive patch test were excluded.

The modelling procedure on age at onset was done twice: first, including all individuals regardless of age; second, including only individuals above 15 years of age. This cut-off was chosen in an attempt to exclude the expected large group with atopic hand eczema among individuals with hand eczema onset in childhood.

Selection of the best fitting model is based on Akaike Information Criterion (AIC)[101;102]. This is defined as -2LL + 2 * q, where -2LL is minus twice log likelihood of data and q is the number of free parameters in the model. The criteria takes into account how well the data fits the model and the degree of parsimony (i.e. increase in the number of parameters in the model is stopped, if this does not lead to a significantly better fit to the data) of the model. The model with the lowest AIC represents the model with the best fit to the data and the most parsimonious model. Nested submodels were compared to the full models using the chi-square ($\chi 2$) test statistic. A high $\chi 2$ and an insignificant p-value indicate that the model offers a good description of the data. Estimation of tetra- and polychoric correlation coefficients and quantitative genetic modelling were done with the software package Mx[103].

4.1.5.2 Manuscript II

The statistical analyses on incidence rates are based on the 3393 individuals *without* self-reported hand eczema in 1996 (individuals at risk). An individual contributed with 8.5 person-years at risk if the individual did not develop hand eczema (from 1 October 1996 to 31 March 2005). Calculation of person-years for individuals who developed hand eczema was based on the year of onset of hand eczema. The year of onset was not reported by 27 individuals; they were assigned the year 2000 (middle of study period) as the year of onset. Incident cases were assumed to develop hand eczema in the middle of the year of onset (i.e. an individual reporting year of onset in 2000 contributed with 3.75 person-years at risk).

The crude incidence rates as well as incidence rates dependent on sex, age, zygosity, atopic dermatitis, positive patch test, wet work, high-risk occupation, smoking and alcohol were determined. By

taking account of changing age during the study period, a twin individual contributed with person-years in more than one age group.

The effect of risk factors on incidence of hand eczema was evaluated with Poisson regression using the command "poisson" in Stata[98]. Effects are presented as incidence rate ratios with 95% confidence intervals. The confidence intervals are corrected for intra-twin correlation using the option "cluster" in Stata. In univariable Poisson regression analyses the effect of sex, age, zygosity, atopic dermatitis, positive patch test, wet work, high-risk occupation, smoking and alcohol was evaluated.

As 759 respondents did not answer the question on job title and to avoid losing too many data, the variable *high-risk occupation* was excluded from the final multiple Poisson analysis. There was a considerable overlap between the variables *high-risk occupation* and *wet work*. Almost 90% answered either yes or no to both wet work and high-risk occupation. All other covariates were included in the multiple Poisson regression model, regardless of statistical significance in the univariable model, due to the predetermined aim of the study. Respondents with missing values in any one of the variables were excluded from the multiple Poisson regression model, thus leaving 3297 individuals for the analysis. Test for interaction was performed for sex and atopic dermatitis against all other variables and for a few other selected combinations.

The incidence rate for twin individuals with and without a co-twin affected by hand eczema was determined separately for MZ and DZ twin individuals. Information on the co-twin's hand eczema status was available in only 2886 twin individuals, restricting the analysis to this group. The co-twin's hand eczema status could change during the follow-up period (i.e. if the co-twin became an incident case). Thus the total number of twin individuals at risk exceeds the number available for analysis as a twin individual could contribute with time at risk in both the analysis of twin individuals with unaffected co-twins and the analysis of twin individuals with affected co-twins. An incidence rate ratio estimating the risk of hand eczema in MZ as opposed to DZ twin individuals with and without a co-twin affected by hand eczema was determined and adjusted for any effect of sex, age, atopic dermatitis, positive patch test and wet work.

4.2 Part 2: The clinical study

4.2.1 Study population and design

In 1997–98, a total of 1076 adult twin individuals participated in a clinical examination, interview and patch testing[1]. The twin individuals were ascertained from the population-based twin cohort of 5610 same-sex twins participating in the questionnaire survey on hand eczema in 1996[14]. If both twins in a twin pair had returned the questionnaire and at least one had reported symptoms of hand eczema or hand eczema, they were invited to participate. In addition, both twin individuals in a twin pair had to live within 60 km of Copenhagen.

In 2005–06, twin individuals participating in the clinical examination in 1997-98 and having self-reported hand eczema themselves or a co-twin with self-reported hand eczema in the questionnaire survey in 1996 were selected to participate in a new clinical examination, interview and patch testing (659 twin individuals). Addresses were obtained from the Danish Civil Registration System of 605 twin individuals. The remaining twin individuals had a protected address, had emigrated or died (see figure 1 for an overview of the recruitment of participants).

A letter describing the aims and methods of the study was sent to the 605 individuals with available addresses. A stamped self-addressed return envelope and a reply card were included. Recipients were asked to return the reply card and tick one of two boxes on the card, marking whether they would agree to participate or not. Individuals who volunteered to participate where requested to write their telephone number(s) on the reply card. One reminder was mailed after one month to non-respondents. Participants were enrolled after informed consent in compliance with the principles of the Helsinki Declaration. All individuals were examined between May 2005 and June 2006.

4.2.2 Clinical examination and interview

Symptoms of hand eczema were recorded and graded using the Hand Eczema Severity Index (HECSI) score[104]. The score is based on registration of objective symptoms and extension. The minimum score is 0 and the maximum score is 360.

In a structured interview participants were asked about self-reported hand eczema, atopic dermatitis, socioeconomic status, age at onset, time of last hand eczema eruption, current exposure to wet work, current glove use and current frequency of hand washing. Individuals with current or previous hand eczema answered questions on occupational and medical consequences.

4.2.3 Patch testing in 2005–06

The invitation letter included written information describing both the patch test procedure and the ready-to-use TRUE Test® system (Mekos Laboratories AS) and instruction on keeping the test material refrigerated. Additionally, individuals willing to participate were contacted personally by telephone. They were given detailed information on the patch test procedure, including risk of severe and flare-up reactions and an appointment at the Dermatological Laboratory was made. In a few cases, individuals were unable to attend to the appointment at the laboratory and these individuals were visited in their homes.

Participants were instructed to place the patches on the upper back three days in advance of the scheduled examination and to mark the location with a pen. The test panels were removed by the participants the day before the appointment in the Dermatological Laboratory. Sun exposure on the back four weeks prior to the patch testing was not allowed and no testing took place during the summer months (July and August). Pregnant and breastfeeding individuals were not tested.

Patch test results were read according to the International Contact Dermatitis Research Group guidelines[4]. A + reaction was defined as homogenous redness and infiltration in the test area, possibly with additional papules. Only + to +++ reactions were considered positive; however, irritant, follicular and doubtful (+?) reactions were also recorded. All readings were done by the author in collaboration with two experienced nurses. Six participants removed an allergen from the test panel before applying the patches, in all cases nickel, due to 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.

4.2.4 Definitions

A diagnosis of *hand eczema* was based on a positive answer to a question on self-reported hand eczema (Have you ever had hand eczema?), given either in the questionnaire survey in 1996 or at the present examination. Participants were asked about *age at onset* of hand eczema at the examination in 1997–98 or in the case of onset in the follow-up period at the examination in 2005–06. *Socioeconomic status* was based on Socio (Statistics Denmark's Socioeconomic classification), 1st edition 1997. This classification system is based upon educational skills. Participants were divided into three groups: (1) Highest/medium level (minimum 15 years of educational training), (2) Basic level (11-14 years of educational training) and (3) Lowest level (up to 10 years of educational training and/or unemployed or retired). *Persistent hand eczema* was defined as hand eczema within the last year prior to the examination in 2005–06. Participants who had their last hand eczema eruption more than one year prior to the examination in 1997–98 were excluded from the analysis on persistence of hand eczema. *Duration* at the first examination was calculated by subtracting year of onset from year at first examination, thus ignoring periods with complete healing in between. Participants were arbitrarily (but due to an aim of evaluating a possible association between long duration and persistence) subdivided into two groups who had had hand eczema $\leq$ 10 years and > 10 years, respectively. *Atopic dermatitis* and *wet work* was defined as described in the questionnaire study.

4.2.5 Data entering

Data were recorded on structured data forms and then typed in twice by the author in an SPSS data editor. Any discrepancies between the first and second typing were checked with the original data form and the correct value was entered.

4.2.6 Genotyping

Buccal swabs or venous blood samples were collected from the participants and kept at -80°C. DNA was prepared from blood samples and mouth swabs using QIAamp -96 DNA procedures (Qiagen GmbH, Hilden, Germany). Genotyping for R501X and 2282del4 was performed by TAQMAN allelic discrimination assays as previously described[76;105].

4.2.7 Control groups

In the analyses on allele frequencies of R501X and 2282del4, allele frequencies in twin individuals with hand eczema were compared with twin individuals without hand eczema, and likewise twin individuals with and without contact allergy and atopic dermatitis were compared. Secondly, allele frequencies in the twin subgroups with hand eczema and contact allergy were compared to a group of 189 children without atopic dermatitis (91 boys and 98 girls) all born to Danish mothers with asthma. The children are currently being followed from birth in a prospective longitudinal follow-up study (the COPSAC study[76;106]). The criteria of Hanifin and Rajka were used to define atopic dermatitis in the COPSAC study[107]. Finally, in an analysis restricted to the subgroup of twins with hand eczema, allele frequencies in the subgroup with and without atopic dermatitis were compared.

4.2.8 Statistical analyses

4.2.8.1 Manuscript III and IV

Data management, descriptive statistical analyses, and the chi squared (χ^2) test (drop-out analysis), were done in SPSS version 13.0[97]. Logistic regression analysis was performed with Stata Statistical Software[98]. All P-values are 2-sided and a 5% significance level was used.

In a multiple logistic regression analysis the potential influence of sex, zygosity, age at onset, socio-economic status, atopic dermatitis and positive patch test in 1997–98 on the risk of sick leave and medical consultations was explored. Likewise the influence of sex, zygosity, age at onset, socioeconomic status, atopic dermatitis, positive patch test in 1997–98, current wet work and duration of hand eczema at the first examination on the risk of persistent hand eczema was evaluated in a multiple logistic regression analysis. The confidence intervals were corrected for intra-twin correlation using the option "cluster" in Stata[98].

4.2.8.2 Manuscript V

The chi squared (χ^2) test was used in the drop-out analysis. When data on both twin individuals in a twin pair was available, one twin individual was randomly excluded from the analysis, thus leaving 183 twin individuals for analysis. Allele frequencies were compared in subgroups of twins and in the COPSAC subgroup using the chi squared (χ^2) test. Both variants were in Hardy-Weinberg equilibrium in the twin cohort, the twin subgroups and in the COPSAC subgroup. SPSS version 13.0 was used for statistical analyses[97].

5 RESULTS

5.1 Part 1: Questionnaire data

5.1.1 Response rate

In total 4128/5048 twin individuals, corresponding to a response rate of 82%, answered the questionnaire in 2005. Participation was declined by 15 individuals: 6 due to health problems other than hand eczema and 9 without a reason; 14 individuals were unable to be traced (both first letter and reminder returned and assigned "recipient unknown at the address"). The respondents comprised 74% and 62% of the twin individuals participating in 1996 and contacted in 1996, respectively.

5.1.2 Drop-out analysis

Compared to respondents in 1996, there was a significantly increased proportion of female respondents ($p<0.001$) and respondents with previously self-reported hand eczema ($p<0.001$). Also, younger individuals were less likely to answer the questionnaire than older individuals ($p=0.004$). There was no statistically significant difference in zygosity between respondents and non-respondents ($p=0.242$) (table 1, manuscript I).

5.1.3 Descriptive statistics and prevalence

Characteristics of all respondents with regard to sex, zygosity, age, age at onset of hand eczema (reported only by 891/979 with self-reported hand eczema) as well as prevalence measures for self-reported hand eczema, atopic dermatitis, positive patch test and frequency of eruptions, are displayed in table 1.

5.1.4 Casewise concordances and correlations

The casewise concordances for self-reported hand eczema for MZ women and men were statistically significantly increased compared to DZ women and men, respectively. A positive correlation (unadjusted and adjusted for atopic dermatitis and age) for MZ women and men, was found, whereas no correlation across DZ twin pairs was found. This difference was statistically significant (table 2, manuscript I).

Concerning frequency of eruptions there was a trend towards higher conditional probabilities for MZ twin pairs than for DZ twin pairs. Further, the polychoric correlations for MZ twin pairs exceeded the correlations across DZ twin pairs; however, this did not reach statistical significance (table 4, manuscript I).

Pearson's correlations in age at onset showed an inconsistent and insignificant pattern with correlations of 0.401 for MZ women and 0.676 for DZ men being the only significant ones (table 2). Casewise concordances for high-risk occupation and wet work were statistically significantly increased for MZ twins compared to DZ twins (table 3).

5.1.5 Quantitative genetic modelling

The best fitting model for self-reported hand eczema, both with and without the covariates included, was a DE-model. With age and atopic dermatitis included in the model, genetic factors explained 41% of the variance in liability and the remaining 59% of the variance in liability was attributable to unique environmental factors (table 3, manuscript I). With respondents reporting a positive patch test excluded from the analyses the estimates changed only marginally (table 4).

Also, the DE-model provided the best fit to the data on frequency of eruptions (table 5); 38% and 31% of the variance was attributable to genetic variance, without and with atopic dermatitis and age included in the model, respectively.

Only data on female twins were included in the quantitative genetic modelling on age at onset because of sparse male data. The AE-model provided the best fit with all female individuals included, whereas an E-model had the best fit when including only female individuals above 15 years in the analysis (table 6 and 7).

5.1.6 Incidence rate and incidence rate ratios

A total of 356 respondents without self-reported hand eczema in the first questionnaire survey reported present or previous hand eczema in the second questionnaire. However, 112 individuals reported a year of onset before 1996; they were regarded neither as incident cases nor as part of population at risk. Thus, the total number of incident cases in the follow-up period was 244. Population at risk constituted 3393 individuals (without self-reported hand eczema at the start of follow-up) and the total number of person-years under observation was 27,843. This yielded a crude incidence rate of 8.8 cases per 1000 person-years (95% confidence interval [95% C.I.] 7.7-9.9).

Incidence rates dependent on sex, age, zygosity (shown only for MZ and DZ), atopic dermatitis, positive patch test, wet work, smoking and alcohol as well as results from the univariable Poisson regression analysis are displayed in table 8. Smoking (regarding both never smokers/current smokers/ex-smokers and pack-years; however, data not shown for pack-years), alcohol consumption, zygosity and age did not have any statistically significant influence on the incidence rate. Female sex, atopic dermatitis, positive patch test, wet work and high-risk occupation were associated with an increased risk in the univariable Poisson regression analyses.

In the multiple Poisson regression (table 8) only atopic dermatitis, positive patch test and wet work remained significant predictors for hand eczema (high-risk occupation not included in the analysis). No statistically significant interaction between variables was detected.

The incidence rates in MZ and DZ twin individuals with co-twins *with* and *without* hand eczema are displayed in table 2, manuscript II. MZ twin individuals with a co-twin with hand eczema had an increased risk of hand eczema compared to DZ twin individuals with an affected co-twin (incidence rate ratio [IRR] 2.4, 95% confidence interval 1.4-4.1) adjusted for sex, age, atopic dermatitis, posi-

tive patch test and wet work. This result was statistically significantly different (p=0.007) from the incidence rate ratio determined for MZ compared to DZ twin individuals with non-affected co-twin individuals (IRR 1.0, 95% C.I. 0.7-1.4) (table 2, manuscript II).

5.2 Part 2: Clinical data

5.2.1 Participation rate

A total of 274 twin individuals volunteered to participate in the clinical examination, interview and patch testing, corresponding to a participation rate of 45% and 41.5% when compared to invited individuals (605) and all individuals with self-reported hand eczema in 1997–98 (659), respectively.

5.2.2 Drop-out analysis

Drop-out analysis of twin individuals participating in the second examination (274) versus those where one or both twin individuals had self-reported hand eczema in 1997–98(659) revealed no statistically significant differences regarding sex, zygosity, hand eczema status, co-twins hand eczema status, patch test status or atopic dermatitis status. Year of birth was the only statistically significant factor influencing willingness to participate and younger individuals were less likely to participate than older individuals (table 9).

5.2.3 Hand eczema and descriptive data

At the first examination in 1997–98, 167 individuals had self-reported hand eczema; however, 15 denied having hand eczema at the second examination. Some had other diagnoses such as psoriasis and polymorphic light eruption on the hands. At the second examination 188 individuals reported hand eczema; however, a subgroup of the incident cases (22/36 individuals) specified a year of onset before the first examination. All but one of these 22 individuals reported or exhibited one or more objective symptoms of hand eczema at the first examination; accordingly they were counted to the group with self-reported hand eczema at the first examination. Taking these exceptions into account, the number of individuals with hand eczema at the first examination was 174 and the number of new cases at the second examination was thus 14. Thus the total number of individuals with hand eczema in 2005–06 was 188.

Descriptive data on the group with hand eczema (N=188) are displayed in table 10. The mean follow-up period was 8.6 years (range 7.4-9.4). Erythema and scaling were the most frequently encountered clinical symptoms and fingers (excluding fingertips) and palms were the most often affected (see figures 1 and 2, manuscript III).

5.2.4 Occupational and medical consequences

The number of individuals having ever been on sick leave because of hand eczema, duration of the sick leave (sum of all periods) as well as the number of individuals who had changed their job

and/or notified the Danish National Board of Industrial Injuries Registry is displayed in table 10. Being in the group with the lowest socioeconomic status and atopic dermatitis were the only statistically significant factors influencing the risk of ever having been on sick leave (OR=5.6; 95% C.I. 1.4-22.3 and OR=2.9; 95% C.I. 1.0-8.1) (table 1, manuscript III).

See table 10 concerning number of medical consultations reported by the participants. Atopic dermatitis was associated with an increased risk of reporting more than one medical consultation compared to no or just one medical consultation (OR=3.0; 95% C.I.1.4-6.4) (table 1, manuscript III).

5.2.5 Persistent hand eczema

Of those with self-reported hand eczema within one year prior to the first examination (N=142), a total of 96 (67.6 %) still had hand eczema within the last year prior to the second examination. Participants with duration of hand eczema > 10 years at the start of follow-up had an increased risk of persistent hand eczema (OR 2.5; 95% C.I. 1.0-6-0). Further, zygosity (DZ) was associated with an increased OR in the analysis (OR=2.6; 95% C.I. 1.2-5.4) (table 2, manuscript III).

5.2.6 Patch test results

In 1997–98 a total of 65/274 individuals (23.7%) had one or more allergies. Two allergies were detected in 16/274 (5.8%) and 3/274 (1.1%) individuals had three positive patch tests. At the second patch testing, 74/270 (27.4%) individuals had at least one positive patch test and 20/270 (7.4%) had two positive patch tests. The frequency of a positive patch test was 9/90 (10%) and 65/180 (36.1%) in men and women, respectively. None had more than two positive reactions at the second patch testing. The frequency of contact allergy in individuals with and without hand eczema was 59/185 (31.3%) and 15/85 (17.6%), respectively. See table 1, manuscript IV for a list of previous and present positive reactions as well as number of lost and new allergies and the distribution of contact allergy in individuals with and without hand eczema. Nickel allergy was the most prevalent allergy, followed by contact sensitivity to thiomersal and fragrance mix. Overall 64/87 (74%) of the positive reactions in 1997–98 were reproduced at the second patch testing in 2005–06. The highest persistence was found for +++ reactions (100% (10/10)), whereas 69% (29/42) and 71% (25/35) of the ++ and + reactions persisted.

5.2.7 Filaggrin null alleles

DNA genotyping was successful in 263 individuals; 183 twin individuals (70 MZ, 103 DZ and 10 with unknown zygosity) were selected for analyses. Descriptive data on the 183 individuals are displayed in table 11.

The overall allele frequencies of R501X and 2282del4 in the twin cohort were 3.3% for both variants (yielding carrier frequencies of 6.6%). As there were no compound heterozygotes or homozygotes the combined carrier frequency was 13.1%. Allele frequencies in the twin cohort, the twin subgroups and the COPSAC subgroup are shown in table 1, manuscript V.

No association between the phenotype with hand eczema and the two variant alleles was found (p=0.828). Further, no association between contact allergy (positive patch test) and the variant alleles could be demonstrated (p=0.600). In the twin subgroup with atopic dermatitis the combined carrier frequency was increased (23.1%) but this did not reach statistical significance when compared to twin individuals without atopic dermatitis (p=0.104).

Allele frequencies in the twin subgroups with hand eczema or contact allergy were not statistically significantly different from allele frequencies in the COPSAC subgroup of children without atopic dermatitis (p=0.152 and p=0.116, respectively). Comparison of the twin subgroup with atopic dermatitis with the COPSAC subgroup reached statistical significance (OR=3.5, 95% C.I. 1.2-10.0, p=0.015).

In the subanalysis, restricted to twin individuals with hand eczema, comparison of the combined allele frequency in individuals with atopic dermatitis (23.1%) with the subgroup without atopic dermatitis (10.1%) was borderline statistically significant (p=0.080).

6 DISCUSSION

6.1 General discussion

6.1.1 Part 1: Questionnaire study

6.1.1.1 Heritability of hand eczema

This study confirmed that genetic risk factors significantly influence the risk of hand eczema. In the previous twin study on hand eczema the heritability estimate was larger and less precise than that estimated in this study (0.65; 95% C.I. 0.33-0.93[14] vs. 0.45; 95% C.I. 0.33-0.57 when unadjusted for atopic dermatitis and age). The difference cannot be explained by a difference in diagnostic criteria as the same question was used to define a case of hand eczema in both surveys. The study population was the same, except for individuals lost to follow-up. However, different statistical approaches were used in the two studies and the quantitative genetic modelling procedure used in the present study may give a more correct estimate[101]. The overall increase in age in the study population may also influence the heritability estimate. Possibly, the environmental variance increase with age and the importance of genetic factors diminishes. This has been observed for other diseases too[108]. The heritability estimate is based on the lifetime prevalence of hand eczema. In the first twin study on hand eczema the lifetime prevalence was 17.4%, whereas in the present survey the lifetime prevalence was 23.7%. This increase is explained by the new incident cases and by an overrepresentation of individuals with previous hand eczema among the respondents. The increased number of cases adds to the accuracy of the heritability estimate.

Furthermore, this study showed that the heritability of hand eczema is independent of atopic dermatitis. A minor decrease in the relative importance of genetic factors was seen when adjusting for atopic dermatitis in the analysis. The determined prevalence of atopic dermatitis is comparable to previously reported estimates in western European countries[16;109], though, considering the adult study population, probably slightly overestimated.

Due to the questionnaire-based diagnosis of hand eczema, exact subclassification into different subtypes of hand eczema was impossible. Because of limited statistical power it was not possible to include further covariates in the quantitative genetic modelling. Indirectly, we tried to assess any effect of contact allergy on the variance estimates by omitting individuals reporting a positive patch test. A total of 10.1% reported a previous positive patch test, which is considerably lower than the population-based estimates of 15-20% reported in a previous study. However, that result was based on obligatory patch testing of all individuals in a population-based cohort irrespective of symptoms[110]. Omission of individuals reporting a positive patch test did not change the heritability estimate. It is questionable whether exclusion of individuals reporting a positive patch test really reflects exclusion of individuals with allergic contact dermatitis on the hands, but if so, then the heritability of hand eczema could be argued to primarily relate to hand eczema due to irritant contact dermatitis. A few studies support the possibility of genetic susceptibility to irritant contact dermatitis[74;75].

The best-fitting model was a DE-model (table 3, manuscript I). However, a DE-model is biologically very unlikely as non-additive interaction of alleles at the same locus contributes to resemblance between full siblings only and not between other relatives[111]. The limited sample size and the resulting lack of statistical power is a plausible cause. In a larger cohort the best fitting model might have been an ADE-model (estimates are equal in the DE-model and the ADE-model).

Genetic factors had less importance when looking at frequency of eruptions, thus the size of the estimated genetic variance component was 0.31. This indicates that treatment and avoidance of irritant and allergenic exposure are important for the prognosis of hand eczema.

The mean age at onset was 24.3 years. Almost 20% reported an age at onset < 15 years and of these approximately 50% had atopic dermatitis. It has previously been shown that individuals with hand eczema due to atopic dermatitis have an earlier debut[7] and in individuals with age at onset below 20 years a total of 63% had atopic dermatitis in another study[24]. The quantitative genetic modelling on age at onset was hampered because of lack of data. Only female individuals concordant for hand eczema were included in the analysis. When all female individuals were included genetic factors explained 37% of the variance in age at onset. Considering the many individuals with concomitant hand eczema and atopic dermatitis when the age at onset was < 15 years, a second analysis was done including only female individuals above 15 years of age. In this model environmental factors alone explained the variance in age at onset. Caution is needed when interpreting the results: the model is based on few cases and confidence intervals are wide. Further studies are needed before any firm conclusions on this subject can be drawn.

6.1.1.2 Prevalence and incidence

The point- and 1-year prevalence estimates determined in this study are comparable to the highest estimates from previous population studies[13;23], whereas the lifetime prevalence exceeds previously reported estimates[24;25]. The question used to define hand eczema was the same in the other two studies reporting lifetime prevalence of hand eczema; however, the investigated cohorts included individuals with a wider age span, 20–65 years and 20–77 years, respectively, compared to an age span of 28–52 years in the present study. As hand eczema occurrence peaks in young adults this may explain some of the difference, and concerning lifetime prevalence older individuals may not be able to recall episodes with hand eczema at a younger age. Additionally, overrepresentation of individuals with previous self-reported hand eczema and female individuals among respondents may have increased the estimate in the present study.

The overall incidence rate of 8.8 cases per 1000 person-years found in this study is slightly higher than previously reported estimates on the incidence rate of hand eczema in the general population (7.9 and 5.5 cases per 1000 person-years, in Holland and Sweden, respectively[5;24]). No comparable Danish data on the incidence rate of hand eczema exist.

Atopic dermatitis, positive patch test and wet work were confirmed statistically significant risk factors for hand eczema. Female sex was a risk factor in the univariable Poisson regression analysis,

but the effect disappeared in the multivariable analysis. A sub-analysis showed that inclusion of wet work explained the difference, which confirms that skin exposure and not female sex itself increases the risk of hand eczema[44]. There was an insignificant trend towards decreased risk with increasing age, which is also in line with a previous study[24] and with studies reporting the highest 1-year prevalence in young adults[21;23]. In a population-based cross-sectional Swedish study, smoking (>5 cigarettes/day) was associated with an increased risk of reporting hand eczema within the last year[25]. Further, in a cross-sectional study on automobile production machine operators, current smoking was associated with an increased risk of current dermatitis[47]. A study on vesicular palmar hand eczema likewise found that smoking was a significant risk factor[46]. A fourth study, comparing the number of cigarettes smoked in groups with and without hand eczema, however, did not find any statistically significant difference[36]. Two studies have indicated that smoking might be a risk factor for contact allergy[112;113]. In this study, no association between smoking status and hand eczema was found. Neither was there any significant association between alcohol consumption and hand eczema. No other studies evaluating alcohol as a potential risk factor for hand eczema are available.

In the analysis comparing the incidence rate in twin individuals having a co-twin *with* hand eczema with the incidence rate in twin individuals having a co-twin *without* hand eczema, the importance of hereditary factors in the aetiology of hand eczema was confirmed with a new method. This analysis was also adjusted for atopic dermatitis, positive patch test, age, sex and wet work.

6.1.2 Part 2: Clinical study

The twin design was not utilized in the clinical study. Different measures were taken in the statistical analyses to account for dependency within twin pairs (see materials and methods).

6.1.2.1 Clinical characteristics and consequences

Erythema and scaling were the most frequently encountered clinical symptoms, which corresponds with previous reports[3;84]. The most frequently affected areas were palms and fingers, which is supported by some studies[114;115], but not others[19;80;116]. However, some of these studies are based on self-reported symptoms[19;80]. Severity was generally mild as judged by the HECSI score, but considerable variation was seen. A number of different severity scores have recently been developed, but none has yet gained general acceptance[117;118]. Recently, a study on sexual dysfunction in Turkish patients with chronic hand eczema using the HECSI score was published[119]. Mean HECSI scores between 27.0 and 53.5 in groups differing with regard to sex and ± depression were reported. However, recruitment of patients is not described, thus a comparison to the present study is difficult. No other studies reporting use of the HECSI score have been found.

The proportion having ever been on sick leave and/or reporting job change due to hand eczema varies widely among different studies, reflecting among other things a variable length of follow-up,

difference in study populations, variation in social insurance systems and the state of the labour market. Between 6% and 48% had reportedly been on sick leave and 3% and 44% reported change of job in a 15-year population-based follow-up study and a 12-year follow-up study on occupational hand eczema, respectively[78;82]. In this study sick leave was reported by 12.4%, and 8.5% stated job change due to hand eczema; 10.1% reported that the hand eczema had been notified to the Danish National Board of Industrial Injuries Registry. To our knowledge no data on the proportion of notified cases in a population-based cohort have previously been published. The proportion of notified cases will likely vary greatly between countries, due to differences in insurance systems and registries.

The proportion of individuals who had seen a doctor (62.7%) and the finding that atopic dermatitis was associated with an increased risk of more than one medical consultation is in agreement with data previously reported[86].

Persistent hand eczema defined as hand eczema within the last year was reported by 67.6%. Estimates on persistence (same definition) between 40% and 77% in a population-based study and two studies on occupational hand eczema after 12–15 years of follow-up have been reported[78;80;82]. Compared to these numbers the proportion of individuals with persistent hand eczema in this study seems high and may partly be explained by the shorter follow-up period.

Duration above 10 years and being a DZ twin were statistically significant risk factors for persistent hand eczema, the latter was unexpected. In a study on occupational chromate dermatitis, duration of symptoms for more than 12 months before diagnosis of chromate sensitivity was associated with persistence of dermatitis[79].

6.1.2.2 Patch test results

The frequencies of positive patch test reactions in 17.6% and 31.3% of individuals without and with hand eczema found in this study are comparable to previous Danish reports. Nielsen et al determined the frequency of contact allergy in the general population in 1990 and 1998 and found estimates of 15.9% and 18.6%, respectively[110], and Mortz et al patch tested unselected school children and found a prevalence of contact allergy of 15.2%[40]. In a recent population-based Norwegian study, at least one positive patch test was found in 26.3%[113]. Meding et al found that 32% of hand eczema patients recruited from the background population had a positive patch test[83]. In a study on occupational hand eczema 65% of all women and 48% of all men had contact allergy[6].

An excess frequency of positive reactions to nickel, thiomersal and fragrance mix as well as sensitization to preservatives, colophony, cobalt dichloride, thiuram mix, p-Phenylenediamine and balsam of Peru explained the higher frequency of positive reactions in individuals with previous or present hand eczema. The higher frequency of contact allergy in women is well known and is primarily due to a high frequency of nickel and fragrance allergy in women[110].

The persistence of contact sensitivity even after many years was confirmed in this study, as 74% of previous positive reactions remained positive. Other studies retesting individuals with contact allergy after a variable time period (3-12 years), have reported persistence rates of positive reactions between 66% and 86%[120-122].

6.1.2.3 Filaggrin null alleles

The combined carrier frequency of the null alleles in the twin cohort (13.1%) was higher than previously reported combined carrier frequencies of between 5.1% and 9.6% in the background population[76;123-126]. However, the higher frequency in the twin cohort is explainable as the cohort was highly selected and had a higher than normal prevalence of hand eczema and atopic dermatitis. We did not find a higher mutation frequency in twin individuals with hand eczema or contact allergy than in twin individuals without hand eczema or in children from the COPSAC subgroup.

The association with atopic dermatitis was confirmed when alleles frequencies in the twin subgroup with atopic dermatitis were compared to the COPSAC subgroup. Comparison of allele frequencies within the twin subgroup with hand eczema revealed a borderline statistically significant association with individuals with atopic dermatitis. This could indicate that the null alleles are a potential marker for increased risk of hand eczema in individuals with atopic dermatitis. It has previously been shown that the risk of hand eczema increases with the severity of atopic dermatitis[34]. However, this hypothesis needs further investigation in a study also including individuals with atopic dermatitis but without hand eczema. In the present study all individuals with atopic dermatitis also had hand eczema.

6.2 Methodological considerations

6.2.1 Part 1: The questionnaire study

6.2.1.1 Validity of questionnaires on hand eczema and atopic dermatitis

Questions on hand eczema have been thoroughly validated. Smit et al compared both a symptom-based and a self-reported diagnosis with a medical diagnosis of hand eczema made by a dermatologist[10]. The symptom-based diagnosis was inspired by Rycroft and Conraads and relied on reports of symptoms (vesicles, papules, pustules, vesicles or exudation or the presence of two or more of the symptoms: erythema, scaling, oedema, fissuring and lichenification), which should have been recurrent or have persisted for more than 3 weeks[127;128]. The symptom-based diagnosis detected all cases with hand eczema (sensitivity 100%); however, the specificity was only 64%. Conversely the sensitivity of the self-reported question ("According to your own opinion, have you suffered from hand dermatitis in the past 12 months?") was 65% and the specificity was 93%. Other studies validating the self-reported question likewise found a moderate or low sensitivity and high specificity[11;12].

Svensson et al investigated to what degree participants were able to identify the clinical symptoms of hand eczema. They found that respondents had difficulties in identifying the symptoms of hand eczema (i.e. erythema, vesicles, papules etc) (κ-values between 0.53-0.65)[129]. In addition, Berg found a poor correlation between the report of skin symptoms and the presence of objective signs[130].

Reliability of the question on self-reported hand eczema was tested by Brisman et al, who sent two identical questionnaires 5 months apart and found a κ-value of 0.79 for the question "Do you have or have you had hand eczema?"[26].

The question on self-reported hand eczema has not been validated in a twin population. Possibly different results on sensitivity and specificity would be obtained, as an over-reporting due to increased awareness in families, in this case the twin pairs, can be anticipated. Should this occur, this would probably not be dependent on zygosity and thus would not influence the heritability estimate. No sex specific calculations on the sensitivity and specificity have been found.

The diagnosis of atopic dermatitis was based on the UK Working Party's Diagnostic Criteria. These criteria have been thoroughly validated, though mostly but not entirely in children[93;131]. In Scandinavian surveys two other questions have traditionally been used to assess atopic dermatitis[95]: a question on flexural eczema ("Have you ever had eczema on the fronts of elbows or behind the knees?") and a question on self-reported atopic dermatitis ("Have you had childhood eczema?"). The latter question was recently validated in a Swedish population and the authors found that the question overestimated the prevalence by a factor of 1.6[132]. Both questions were included in the present questionnaire and the resulting prevalences were 12.0% (flexural eczema) and 6.7% (childhood eczema). In another study factors influencing recollection of atopic dermatitis in childhood were investigated in adults aged 31–42 years[133]. Almost one third of the cases did not report that they had had atopic dermatitis when asked about previous childhood eczema. Cases with greater disease severity and disease activity after the age of 15 years were more likely to report childhood atopic dermatitis.

The reliability of a question on onset of hand eczema has been evaluated in 35 people with hand eczema[26]. Two identical questionnaires were mailed five months apart. Identical answers were given by 11 respondents; in 12 respondents the answer differed by one year, in 6 by two years, in 3 by three years, in 1 by six years and in 2 by seven years.

Concerning validity of questionnaire data on exposure one study found that duration of wet work was overestimated by a factor two, whereas the frequency of exposure to wet work was underestimated by the same factor[134]. However, a recent study found a strong correlation between self-reports and observations for questions on exposure to water, foodstuffs and occlusive gloves and also a moderate correlation for questions regarding frequency of hand washing[135]. Job titles however, have been shown not to be a good proxy for exposure[136].

The questions on frequency of eruptions have not been validated, thus conclusions on this variable should be drawn with caution.

6.2.1.2 Validity of twin studies

Extrapolation of the results to the general population requires that the twin population is representative of the background population. The study population was drawn from twin cohorts in the Danish Twin Registry, which is based on the Danish Civil Registration System and covers 74.4% of all twins born 1953–67 (incl.) and 97.4% of those born 1968–1982 (incl.)[137;138]. Further, it has been shown that the Danish twin cohort is representative of the Danish population in terms of many common diseases and mortality rate[139;140], and the prevalence measures obtained in this study are comparable to estimates from non-twin populations and were independent of zygosity.

A second important assumption in the classical twin study is the one of equal environment. MZ twins are assumed to share the environment to the same degree as DZ twins. If this is not the case and MZ twins experience a more similar environment than DZ twins, then a greater phenotypic similarity between the MZ twins than among the DZ twins is not only due to a greater genetic similarity but also to a greater environmental similarity. In that case the effect of genetic factors will be overestimated. A series of studies testing and supporting the assumption has been published and is summarized by Kyvik[140].

We found statistically significantly higher concordances for high-risk occupation and exposure to wet work for MZ than for DZ twins, thus it seems that the MZ twins choose more similar occupations than the DZ twins. This may potentially have inflated the heritability estimate.

The third crucial assumption in twin studies concerns the biology of twinning with MZ twins arising from one ovum and DZ twins resulting from a double ovulation. Although, rare exceptions (genetically discordant MZ twins and DZ twins with different fathers) are reported, the explanation of twinning as described above is valid in the vast majority of cases[140]. Epigenetic changes (i.e. DNA modifications, which change the expression of a sequence of DNA, but without changing the DNA sequence), however, challenge the assumption of identical genes in MZ twins. Thus, some of the phenotypic differences in MZ twins could result from their epigenetic differences[141;142]. It is suggested that epigenetic modifications can be due to stochastic events or due to external environmental factors[143]. It has been shown that older twins were epigenetically more dissimilar than younger twins and twin pairs who had spent less of their lifetime together were epigenetically more different than twin pairs with a higher percentage of lifetime shared[142].

Correct zygosity determination is essential in the classical twin study. Zygosity determination in the Danish Twin Registry relies on the questionnaire-based similarity method[138]. The overall misclassification using this method has been shown to be 4%, with MZ twins having the highest misclassification[144]. The influence of such a misclassification on the twin analyses would be a slight underestimation of the effect of genetic factors.

Finally, the quantitative genetic model procedure assumes random mating, no gene-environment interaction or correlation. Non-random mating is mating characterized by a tendency to choose a partner with similar traits, which is unlikely in the case of hand eczema. In a twin study, non-random mating will increase the similarity between DZ twins relative to MZ twins and will thus tend to decrease the heritability estimate[101]. Non-random mating is usually highest with respect to traits such as education and socioeconomic status[101], factors which may be associated with differences in hand eczema risk; however, we hypothesize that non-random mating is not a significant problem in this study on hand eczema.

Different genotypes respond differently to the same environment and this is called gene-environment interaction[101]. Gene-environment correlation refers to the fact that individuals seek environmental exposure or create specific environments as a function of their genotype[101]. We did not examine gene-environment interaction in this study and the extent to which gene-environment interaction and correlation potentially influence the result in this study is unknown.

6.2.1.3 Evaluation of risk factors in the Poisson regression

In the Poisson regression associations between potential risk factors and hand eczema were explored. However, apart from the variables sex, age and zygosity, information on exposure (atopic dermatitis, contact allergy, wet work, high-risk occupation, smoking history and alcohol consumption) was obtained after disease occurrence. Preferably, exposure information should have been collected at the start of follow-up. However, this kind of information was not available and thus any conclusions regarding cause and effect are hampered. Additionally, exposure may have changed during the follow-up period and may not even have been present when hand eczema developed, thus the results should be interpreted with caution.

6.2.1.4 Bias and confounding

The overrepresentation of women and individuals with previously self-reported hand eczema among respondents may have caused selection bias and increased the estimates of disease occurrence. However, this effect was counteracted by the fact that younger individuals were less likely to participate.

Information bias on the diagnosis of hand eczema (due to low sensitivity) may have underestimated the occurrence and risk estimates[11]. The possible misclassification, which is likely of the same size in MZ and DZ individuals, will tend to lower the heritability estimate.

Misclassification of atopic dermatitis due to recall bias may result in an underestimation of the occurrence, an underestimation of the risk estimate in the Poisson regression and of the potential effect of atopic dermatitis on the heritability estimate of hand eczema. However, the prevalence estimate of atopic dermatitis did not seem to be underestimated. Individuals with hand eczema may recall previous atopic dermatitis to a higher degree than individuals without hand eczema and this would then increase the risk estimate obtained in the Poisson regression.

Answers regarding wet work may also be influenced by information bias. Possibly, individuals with hand eczema are more conscious of harmful skin exposure and thus report this to a higher degree than individuals without hand eczema, irrespective of an equal exposure. In that case the risk estimate in the Poisson regression will be overestimated.

The natural history of hand eczema is characterised by disease-free intervals and more or less frequent recurrent eruptions. With more than eight years of follow-up relapses can be confused with debut and respondents with a short eruption of hand eczema at the beginning of the follow-up period may forget about it. Because of the relatively short follow-up period, we do not believe that it is an important problem in this study.

Respondents with hand eczema will tend to have a patch test performed more often than respondents without hand eczema. Also, respondents with hand eczema may be able to recall their (positive) test result to a higher degree than respondents without hand eczema. These very likely biases may have considerably inflated the risk estimate of contact allergy.

In the quantitative genetic modelling the hypothesis of atopic dermatitis being a possible confounder in the analysis on heritability of hand eczema was explored and to date rejected. In the Poisson regression, the association between female sex and hand eczema disappeared when wet work was included in the analysis, thus, wet work was a confounder for the association between sex and hand eczema.

6.2.2 Part 2: The clinical study

6.2.2.1 Diagnosis of hand eczema

The diagnosis of hand eczema in the clinical study relied on a self-report of hand eczema. The sensitivity and specificity of this question has already been discussed. Misclassification, especially mild cases misclassified as not having hand eczema and thus not being invited to participate, is likely. Supporting this, studies evaluating the question on self-reported hand eczema have shown that respondents with false negative answers usually have mild or moderate symptoms[43;145]. Cases without hand eczema misclassified as having hand eczema may have occurred; however, symptoms of hand eczema at either one of the examinations were present in 56.9% of all participants. The remaining individuals had historic symptoms, which rendered a clinical diagnosis impossible.

Misclassification was evident as some of the participants previously reporting hand eczema denied having hand eczema at the present examination and also some incident cases reported a year of onset before the second examination.

6.2.2.2 The patch test procedure

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. Even though the participants were carefully instructed and questioned in detail upon examination, errors and inconsistencies in the test procedure cannot be excluded. Secondly, as reading was done only after 72 hours, late reactions may have been missed and the frequency of positive reactions may be underestimated. It has been demonstrated that between 3% and 8.2% of reactions become positive on day 6 or 7[146;147].

Individuals were tested with 20 allergens only, including the most frequent sensitizers. Thus, a negative test is obviously not the same as absence of contact allergy. However, the European standard test series, which is similar to the TRUE Test®, has been shown to detect 75%–80% of all contact allergies in departments specialized in contact dermatitis[148]. Two different investigators read all patch test reactions. However, both were educated in the same department and both were experienced with the patch test procedure.

6.2.2.3 Bias

The participation rate in the clinical study was unexpectedly low. Possible explanations include unwillingness to repeat the patch test and moving to another geographical area.

Drop-out analysis revealed no statistically significant difference between participants and non-participants concerning sex, zygosity, hand eczema status, co-twin's hand eczema status, patch test status or atopic dermatitis status, thus selection bias is less likely to have influenced the results. However, an increased tendency for individuals with recurrent or more severe symptoms of hand eczema or allergic contact dermatitis to attend cannot be excluded. Thus, the proportion with persistent symptoms, occupational and medical effects and a persistent positive patch test may be increased in the sample and the analyses on factors associated with persistence may be hampered by loss to follow-up of already recovered cases. Supporting this, a previous study found that more participating individuals had continuous symptoms than did those not participating[149].

Recall bias may influence the results, as information on occupational and medical effects, age at onset, year of last eruption and atopic dermatitis was based on questions answered by the participants. The effect of a potential recall bias is more unpredictable and both under- and overestimation of the estimates is possible.

Interviewer or observer bias is a theoretical possibility as hand eczema status was unblinded to the investigator. Any tendency of the interviewer or observer to record potential risk factors in individuals with hand eczema more often than in non-affected individuals would tend to increase the risk estimates. As the interview was highly structured and strict guidelines concerning patch test reading were followed, any influence of such bias is thought to be negligible.

6.2.2.4 Control group in analysis of allele frequencies

The COPSAC subgroup of children without atopic dermatitis was chosen as a secondary control group due to the limited number of twin individuals without hand eczema and due to availability. As some of the children will develop hand eczema and contact allergy later in life, the COPSAC subgroup of children is a suboptimal control group. A more suitable control group would be an adult group without hand eczema and contact allergy, matched on sex and atopy status.

7 CONCLUSIONS

Part 1: Questionnaire study

Heritability of hand eczema is not explained by comorbidity with atopic dermatitis. Genetic factors, independent of atopic dermatitis, explain 41% of the phenotypic variance in liability to develop hand eczema. Concerning frequency of eruptions, 31% of the phenotypic variance is explained by genetic factors, underlining the importance of treatment and secondary prevention.

The incidence rate of hand eczema in this population-based adult cohort is 8.8 cases per 1000 person-years, which is slightly higher than previously reported estimates from population-based studies in Sweden and Holland. No previous Danish population-based incidence data are available for comparison.

Atopic dermatitis, positive patch test and wet work are confirmed risk factors for hand eczema as indicated by an increased incidence rate in individuals reporting these factors. Female sex was associated with an increased risk of hand eczema, which was explained by a higher frequency of wet work in women. This confirms that skin exposure and not female sex itself increases the risk of hand eczema. Smoking and alcohol were not associated with an increased risk of hand eczema in this study.

Part 2: Clinical study

The most frequent symptoms of hand eczema were erythema and scaling with palms and fingers being involved the most often. Symptoms were generally mild, although considerable variation in severity was seen. Sick leave and job change ever due to hand eczema affected 12.4% and 8.5%, whereas 10.1% reported notification to the Danish National Board of Industrial Injuries; 62.7% reported seeing a doctor at least once because of hand eczema. Low socioeconomic status and atopic dermatitis were risk factors for sick leave, whereas atopic dermatitis was a risk factor for more than one medical consultation. Persistence of hand eczema (hand eczema within the last year) after 8.6 years of follow-up was reported by 67.6%. Duration > 10 years at the start of follow-up was a risk factor for persistence of hand eczema. A total of 74% of previous positive patch test reactions were persistent positive at follow-up and a higher frequency of positive patch test reactions was found in individuals with hand eczema. No association between the filaggrin null alleles and hand eczema or contact allergy was found.

8 PERSPECTIVES AND FUTURE STUDIES

The statistical evidence of heritability of hand eczema remains tentative until identification of specific susceptibility genes is documented. Thus a search for and possibly identification of genetic markers increasing the risk of hand eczema is an obvious next step. Searching for candidate genes for hand eczema as a single clinical entity may be an impossible task, due to the heterogeneity of the disease, both concerning aetiology, morphology, severity and prognosis. A careful characterization and selection of individuals with different phenotypes, i.e. different subtypes of hand eczema, is required in future studies addressing the heritability of hand eczema and associations between specific genetic polymorphisms and hand eczema. This characterization of subtypes and different phenotypes is a real challenge as no "gold standards" exist.

The ultimate objective of understanding the role of genetic factors in the aetiology of hand eczema is prevention and treatment. If individual susceptibility genes for hand eczema or subtypes of hand eczema are recognised, individuals with increased risk may be identified and instructed on precautions. Further, genetic markers of hand eczema or subtypes of hand eczema could possibly be of diagnostic and prognostic help. For the patient and the clinician this would be an important improvement.

Even though hand eczema has been a research subject for years and important risk factors have been identified, prevalence and incidence measures obtained in this study did not indicate a decrease in occurrence. Further, the prolonged persistence of symptoms in many individuals and the occupational and medical consequences of hand eczema documented in this thesis, illustrate the substantial burden on health, both from a societal and individual point of view caused by hand eczema. Primary and secondary evidence-based prevention of hand eczema seems of unchanged importance. Atopic dermatitis and low socioeconomic status were significant risk factors for sick leave due to hand eczema. These concrete findings may help the physician to identify individuals with increased need of special attention.

The importance of the filaggrin variant alleles in the pathogenesis of atopic dermatitis is indisputable. Although, no association between the variant alleles and hand eczema or contact allergy was demonstrated in the present study, the question of a possible importance of the filaggrin variant alleles in the context of hand eczema is not exhausted. A role in the pathogenesis and prognosis of atopic hand eczema is likely. In addition, the possible role of the variant alleles and the resulting impaired skin barrier in the context of contact allergy in conjunction with atopic dermatitis would be interesting to explore further.

9 SUMMARY

Hand eczema is a frequent disease with a considerable risk of chronic symptoms, sick leave and job change. Preventive measures require insight into the pathogenesis of hand eczema and risk factors for development. The aims of this thesis were to investigate the relative importance of genetic and environmental risk factors for hand eczema independent of atopic dermatitis; to determine the incidence rate of hand eczema in a population-based adult cohort and assess possible risk factors for hand eczema; to investigate the clinical characteristics, including patch test reactivity, and consequences of hand eczema; and to investigate a possible association between the filaggrin null alleles, R501X and 2282del4, and hand eczema or contact allergy.

The thesis includes results from two separate, but related projects. Both projects are follow-up studies on a twin study on hand eczema from 1996–99. The first study was a population-based questionnaire survey including 4128 twin individuals born between 1953 and 1976 who previously participated in a questionnaire survey in 1996. Secondly, a subgroup of 274 twin individuals with self-reported hand eczema or a co-twin with self-reported hand eczema participated in a clinical examination, patch test and interview. These individuals participated in a similar examination in 1997–98. All individuals were ascertained from the Danish Twin Registry.

The heritability of hand eczema independent of atopic dermatitis was estimated using quantitative genetic modelling. Genetic factors, independent of atopic dermatitis explained 41% of the variance in liability to develop hand eczema. The crude incidence rate of hand eczema was 8.8 cases per 1000 person-years. Monozygotic twin individuals with a co-twin affected by hand eczema had a doubled risk of hand eczema compared to dizygotic twin individuals with a co-twin affected by hand eczema, confirming the importance of genetic risk factors. In addition, reporting a positive patch test, atopic dermatitis and wet work were significant risk factors.

In the clinical survey, sick leave and job change due to hand eczema was reported by 12.4% and 8.5%, respectively. The majority (62.7%) had seen a doctor because of hand eczema at least once. Low socioeconomic status and atopic dermatitis were risk factors for sick leave due to hand eczema. Persistence of hand eczema after 8 years of follow-up was reported by 67.7%, and long duration (>10 years) at the start of follow-up was a risk factor for persistence of hand eczema. The frequency of contact sensitivity in individuals with and without hand eczema was 31.3% and 17.6%, respectively. No association between the filaggrin null alleles and hand eczema or contact allergy was found. The previously reported association with atopic dermatitis was confirmed.

In conclusion, genetic risk factors independent of atopic dermatitis were shown to have a moderate influence on the risk of hand eczema. Characterization of different phenotypes of hand eczema and a search for possible associated genetic polymorphisms is an interesting, natural next step to exploit this knowledge with regard to prevention, diagnosis and prognosis. A wide spectrum of disease severity, consequences and persistence was found, overall with a tendency to chronic symptoms. Thus the importance of preventive measures is unchanged. No association between the filaggrin null alleles and hand eczema or contact allergy was found. Further association studies in selected hand eczema subgroups are an interesting future research subject.

10 SUMMARY IN DANISH

Håndeksem er en hyppig sygdom med en betydelig risiko for kroniske symptomer samt arbejdsmæssige konsekvenser i form af sygemelding og jobskifte. Viden om risikofaktorer for udvikling af håndeksem er vigtig ved planlægningen af forebyggelsesmæssige tiltag. Formålet med denne afhandling var at undersøge, i hvilken grad genetiske og miljømæssige risikofaktorer for håndeksem uafhængigt af atopisk eksem har betydning for at udvikle håndeksem. Desuden at bestemme incidensraten af håndeksem i den voksne baggrundsbefolkning og undersøge faktorer, som influerer på incidensraten. Dernæst at undersøge kliniske karakteristika ved håndeksem og konsekvenser af håndeksem. Endelig at undersøge en mulig sammenhæng mellem filaggrin variant allelerne, R501X og 2282del4, og håndeksem eller kontaktallergi.

Afhandlingen indeholder resultater fra to separate, men beslægtede studier. Begge studier er opfølgende undersøgelser på et tvillingestudie om håndeksem fra 1996–99. Det første studie var en populationsbaseret spørgeskemaundersøgelse med deltagelse af 4128 tvillinger tilhørende fødselsårgangene 1953–1976, som tidligere havde deltaget i en spørgeskemaundersøgelse i 1996. Dernæst blev en mindre gruppe på 274 tvillinger, som havde håndeksem eller som havde en co-tvilling med håndeksem, undersøgt klinisk, interviewet og lappetestet. Sidstnævnte tvillinger havde alle deltaget i en lignende klinisk undersøgelse i 1997–98. Alle tvillinger var rekrutteret fra Det Danske Tvillingregister.

Arveligheden ved håndeksem blev bestemt ved hjælp af et klassisk tvillingestudie med sammenligning af monozygote og dizygote tvillinger. Genetiske faktorer uafhængigt af atopisk eksem forklarede 41% af variansen i tilbøjeligheden til at udvikle håndeksem. Incidensen af håndeksem var 8.8 tilfælde per 1000 person-år. Monozygote tvillinger, som havde en co-tvilling med håndeksem, havde en dobbelt så høj risiko for at udvikle håndeksem sammenlignet med dizygote tvillinger, som havde en co-tvilling med håndeksem. Betydningen af genetiske faktorer for udviklingen af håndeksem blev således bekræftet. Kontaktallergi, atopisk eksem og vådt arbejde var associeret til en øget risiko for håndeksem.

I den kliniske undersøgelse fandtes, at henholdsvis 12.4% og 5.8% havde været sygemeldt eller havde skiftet job på grund af håndeksem. Flertallet (62.7%) havde været til læge mindst én gang på grund af håndeksem. Lav socio-økonomisk status og atopisk eksem var associeret med en øget risiko for sygemelding på grund af håndeksem. Efter ca. 8 års opfølgning havde 67.7% fortsat håndeksem. Lang varighed (> 10 år) ved starten af opfølgningsperioden var associeret med en øget risiko for vedvarende håndeksem. Frekvensen af kontaktallergi var henholdsvis 31.3% og 17.6% hos individer med og uden håndeksem. Der fandtes ingen association mellem filaggrin nul allelerne, R501X og 2282del4, og håndeksem eller kontaktallergi.

Vi fandt således, at genetiske faktorer uafhængige af atopisk eksem havde en moderat indflydelse på risikoen for at udvikle håndeksem. Yderligere karakterisering af forskellige fænotyper af håndeksem og undersøgelse af mulige associerede genetiske polymorfier er et interessant og nærliggende emne for fremtidige studier. Vi fandt desuden stor variation i sygdommens sværhedsgrad og konsekvenser, dog fandtes en tendens til langvarige symptomer. Forebyggelse har derfor stadig stor betydning.

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12 FIGURE 1 AND TABLES 1-11

Figure 1. Diagram showing recruitment of participants to the clinical examination and patch testing (Part II).

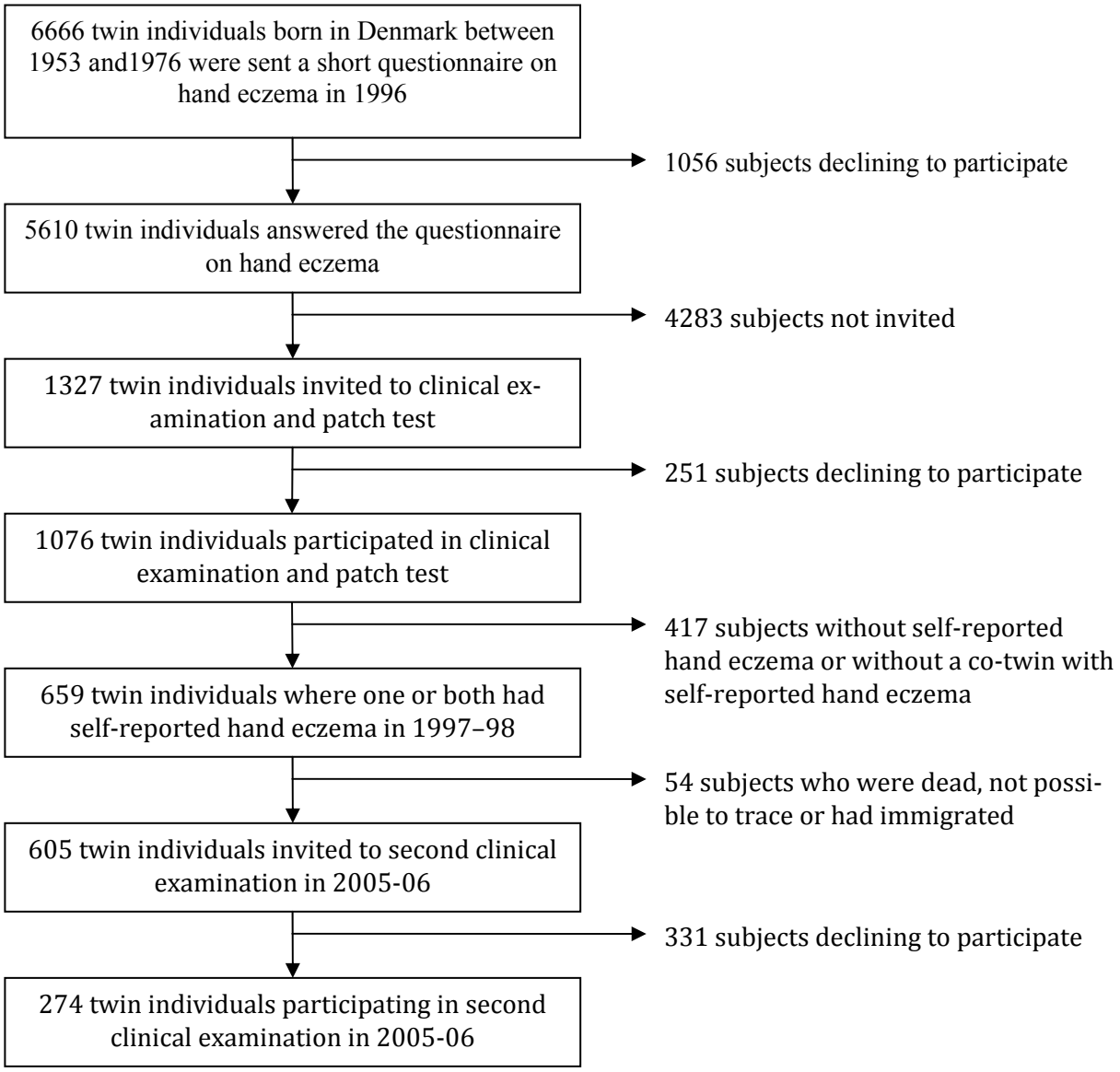

Table 1. Characteristics of respondents (N=4128) and prevalence measures.

Demographics		
Sex (females/males)		59% /41%
Zygosity (MZ/DZ/Tr/Qr/UZ)		1717 / 2198 / 44 / 2 / 167
Mean age		40.4 years (SD 6.6, range 28-52)
Mean age at onset of hand eczema		24.3 (SD 10.5, range 0-51)
Age at onset ≤ 15 years		19.3%
Prevalence measures		**% (95% C.I.)**
Self-reported hand eczema	Point prevalence	5.9 (5.2-6.6)
	1-year prevalence	11.8 (10.8-12.8)
	Lifetime prevalence	23.7 (22.4-25.0)
Atopic dermatitis[1]		16.4 (15.3-17.5)
Atopic dermatitis if age at onset of hand eczema < 15 years		48.3
Positive patch test[2]		10.1 (9.2-11.0)
Frequency of eruptions[3]	One episode	24.9 (22.5-27.9)
	More than one	54.0 (50.5-56.7)
	All the time	16.0 (13.7-18.3)

MZ: monozygotic; DZ: dizygotic; Tr: triplets; Qr: quadruplets; UZ: unknown zygosity.
SD: Standard deviation.
95% C.I.: 95% confidence interval.
[1]UK Working Party's Diagnostic criteria.
[2]Self-reported.
[3]5.1% of individuals with hand eczema did not answer the question.

Table 2. Pearson's correlation coefficients for age at onset of self-reported hand eczema.

	Age at onset (all)	p-value	Age at onset (> 15 years)	P-value
MZM	0.404	0.134	0.156	0.594
DZM	0.676	**0.046**	-0.454	0.546
MZF	0.401	**0.010**	0.026	0.900
DZF	0.164	0.289	0.244	0.210

MZM: monozygotic males; DZM: dizygotic males; MZF: monozygotic females; DZF: dizygotic females.
Statistically significant p-values highlighted in bold.

Table 3. Casewise concordances, prevalence and total number of concordant/discordant pairs concerning high-risk occupation and wet work.
(MZ pairs N=632; DZ pairs N=755).

	Concordant (n)	Discordant (n)	Prevalence	Casewise concordance[1]
High-risk occupation				
MZ	29	111	0.13	0.34 (0.25-0.43)
DZ	21	169	0.14	0.20 (0.13-0.27)
Wet Work				
MZ	53	127	0.18	0.46 (0.37-0.53)
DZ	39	232	0.21	0.25 (0.19-0.31)

MZ: monozygotic; DZ: dizygotic.
95% confidence interval given in parentheses.
Concordant pairs mean that both twin individuals in a twin pair have high-risk occupation/wet work. Discordant pairs mean that one twin in a twin pair has high-risk occupation/wet work.
[1]Statistically significant difference between MZ and DZ for both high-risk occupation and wet work.

Table 4. Results of the quantitative genetic model fitting for self-reported hand eczema, when respondents reporting a previous positive patch test were excluded from the analyses, showing the relative contribution of genetic and environmental components to the total phenotypic variance.

		Genetic variance components		Environmental variance components		-2LL[1]	Comparison of nested submodels			
	Model	A	D	C	E		$\Delta\chi2$[2]	Δd.f.[3]	ΔAIC	P
Adjusted[5]	ADE	0.00	0.42	-	0.58	2076.66	2.34	5	-7.661	0.80
	AE	0.38	-	-	0.62	2078.68	4.36	6	-7.643	0.63[4]
	DE	**-**	**0.42**	**-**	**0.58**	**2076.66**	**2.34**	**6**	**-9.661**	**0.89**
	E	-	-	1	-	2103.60	29.28	7	15.276	0.00[4]
	ACE	0.38	-	0.00	0.62	2078.68	2.49	5	-7.507	0.78
	CE	-	-	0.26	0.74	2085.66	9.48	6	-2.523	0.15

A: Additive genetic component; D: non-additive genetic component; C: common environmental component; E: unique environmental component.
The model with the lowest AIC represents the model with the best fit. Selection of the best-fitting submodel is based on $\Delta\chi2$ and an insignificant P-value. The best-fitting model is highlighted in bold.
[1]Minus 2 times log likelihood of data.
[2]Difference in $\chi2$ between the full model and the submodel.
[3]Difference in degrees of freedom (d.f.) between the full model and the submodel.
[4]Compared to an ADE-model.
[5]Adjusted for age and atopic dermatitis.

Table 5. Results of the quantitative genetic model fitting for frequency of eruptions, showing the relative contribution of genetic and environmental components to the total phenotypic variance.

		Genetic variance components		Environmental variance components		-2LL[1]	Comparison of nested submodels			
	Model	A	D	C	E		$\Delta\chi2$[2]	Δd.f.[3]	ΔAIC	P
Unadjusted	ADE	0.00 (0.00-0.42)	0.38 (0.00-0.50)	-	0.62 (0.51-0.74)	5768.24	0.29	2	-3.713	0.867
	AE	0.34 (0.22-0.45)	-	-	0.66 (0.55-0.77)	5770.36	2.40	3	-3.596	0.493[4]
	DE	-	**0.38 (0.26-0.49)**	-	**0.62 (0.51-0.74)**	**5768.24**	**0.29**	**3**	**-5.713**	**0.963**
	E	-	-	-	1	5801.79	33.83	4	25.837	< 0.001[4]
	ACE	0.34 (0.15-0.45)	-	0.00 (0.00-0.14)	0.66 (0.55-0.77)	5770.36	0.01	2	-3.993	0.997
	CE	-	-	0.23 (0.13-0.31)	0.77 (0.69-0.87)	5778.79	8.44	3	2.444	0.038
Adjusted[5]	ADE	0.00 (0.00-0.36)	0.31 (0.00-0.43)	-	0.6 (0.57- 0.82)	4031.78	0.77	5	2.579	-7.421
	AE	0.28 (0.16-0.39)	-	-	0.72 (0.61-0.84)	4033.38	0.65	6	4.178	-7.822[4]
	DE	-	**0.31 (0.18-0.43)**	-	**0.69 (0.57-0.82)**	**4031.78**	**0.86**	**6**	**2.579**	**-9.421**
	E	-	-	-	1	4053.11	0.01	7	23.900	9.900[4]
	ACE	0.28 (0.06-0.39)	-	0.00 (0.00-0.15)	0.72 (0.61-0.84)	4033.38	0.77	5	2.548	-7.452
	CE	-	-	0.18 (0.09-0.27)	0.82 (0.73-0.91)	4038.92	0.23	6	8.087	-3.913

95% confidence intervals given in parentheses.
A: Additive genetic component; D: non-additive genetic component; C: common environmental component; E: unique environmental component.
The model with the lowest AIC represents the model with the best fit. Selection of the best-fitting submodel is based on $\Delta\chi2$ and an insignificant P-value. The best-fitting models are highlighted in bold.
[1]Minus 2 times log likelihood of data.
[2]Difference in $\chi2$ between the full model and the submodel.
[3]Difference in degrees of freedom (d.f.) between the full model and the submodel.
[4]Compared to an ADE-model.
[5]Adjusted for age and atopic dermatitis.

Table 6. Results of the quantitative genetic model fitting for age at onset of hand eczema, showing the relative contribution of genetic and environmental components to the total phenotypic variance. Only women included in the analysis.

		Genetic variance components		Environmental variance components		-2LL[1]	Comparison of nested submodels			
	Model	A	D	C	E		$\Delta\chi2$[2]	Δd.f.[3]	ΔAIC	P
Unadjusted	ADE	0.42 (0.00-0.61)	0.00 (0.00-0.62)	-	0.58 (0.39-0.84)	4303.44	-	-	-	-
	AE	**0.42 (0.16-0.61)**	**-**	**-**	**0.58 (0.39-0.84)**	**4303.44**	**0.000**	**1**	**-2.000**	**0.999**
	DE	-	0.44 (0.16-0.62)	-	0.56 (0.38-0.84)	4303.62	0.187	1	-1.813	0.666
	E	-	-	-	1	4312.56	9.120	2	5.120	0.010
	ACE	0.42 (0.00-0.61)	-	0.00 (0.00-0.46)	0.58 (0.39-0.85)	4303.44	-	-	-	-
	CE	-	-	0.32 (0.10-0.49)	0.68 (0.51-0.90)	4312.56	1.1412	1	-0.588	0.235
Adjusted[5]	ADE	0.34 (0.00-0.62)	0.10 (0.00-0.63)	-	0.56 (0.37-0.83)	3343.55	16.795	1	14.795	0.000
	AE	**0.37 (0.08-0.58)**	**-**	**-**	**0.63 (0.42-0.92)**	**3326.84**	**0.092**	**1**	**-1.908**	**0.762**
	DE	-	0.39 (0.09-0.60)	-	0.61 (0.40-0.91)	3326.79	0.037	1	-1.963	0.847
	E		-	-	1	3333.00	6.249	2	2.249	0.044
	ACE	0.43 (0.00-0.62)	-	0.00 (0.00-0.47)	0.57 (0.38-0.85)	3342.57	16.723	1	14.723	0.000
	CE	-	-	0.28 (0.04-0.47)	0.72 (0.53-0.96)	3328.02	1.175	1	-0.825	0.278

95% confidence intervals given in parentheses.
A: Additive genetic component; D: non-additive genetic component; C: common environmental component; E: unique environmental component.
The model with the lowest AIC represents the model with the best fit. Selection of the best-fitting submodel is based on $\Delta\chi2$ and an insignificant P-value. The best-fitting models are highlighted in bold.
[1]Minus 2 times log likelihood of data.
[2]Difference in $\chi2$ between the full model and the submodel.
[3]Difference in degrees of freedom (d.f.) between the full model and the submodel.
[4]Compared to an ADE-model.
[5]Adjusted for atopic dermatitis.

Table 7. Results of the quantitative genetic model fitting for age at onset of hand eczema, showing the relative contribution of genetic and environmental components to the total phenotypic variance. Only female individuals > 15 years included in the analyses.

		Genetic variance components		Environmental variance components		-2LL[1]	Comparison of nested submodels			
	Model	A	D	C	E		$\Delta\chi 2^2$	Δd.f.[3]	ΔAIC	P
Unadjusted	ADE	0.24 (0.00-0.66)	0.00 0.00-0.62)	-	0.76 (0.34-1.00)	693.08	-	-	-	-
	AE	0.24 (0.00-0.66)	-	-	0.76 (0.34-1.00)	693.08	0.000	1	-2.000	1
	DE	-	0.16 (0.00-0.64)	-	0.84 (0.36-1.00)	693.55	0.473	1	-1.527	0.492
	E	-	-	-	**1**	**693.74**	**0.660**	**2**	**-3.340**	**0.719**
	ACE	0.00 (0.00-0.63)	-	0.23 (0.00-0.60)	0.77 (0.35-1.00)	692.39	-	-	-	-
	CE	-	-	0.23 (0.00-0.60)	0.77 (0.40-1.00)	692.39	0.000	1	-2.000	1
Adjusted[5]	ADE	0.24 (0.00-0.66)	-	0.00 (0.00-0.62)	0.76 (0.34-1.00)	693.08	6.111	1	4.111	0.013
	AE	0.10 (0.00-0.57)	-	-	0.90 (0.43-1.00)	686.96	0.000	1	-2.000	1.000
	DE	-	0.00 (0.00-0.53)	-	1.00 (0.47-1.00)	687.07	0.107	1	-1.893	0.743
	E	-	-	-	**1**	**687.07**	**0.107**	**2**	**-3.893**	**0.948**
	ACE	0.00 (0.00-0.63)	-	0.23 (0.00-0.60)	0.77 (0.35-1.00)	692.39	5.953	1	3.953	0.015
	CE	-	-	0.16 (0.00-0.52)	0.84 (0.48-1.00)	686.44	0.000	1	-2.000	-1.000

95% confidence intervals given in parentheses.
A: additive genetic component; D: non-additive genetic component; C: common environmental component; E: unique environmental component.
The model with the lowest AIC represents the model with the best fit. Selection of the best-fitting submodel is based on $\Delta\chi^2$ and an insignificant P-value. The best-fitting models are highlighted in bold.
[1]Minus 2 times log likelihood of data.
[2]Difference in χ^2 between the full model and the submodel.
[3]Difference in degrees of freedom (d.f.) between the full model and the submodel.
[4]Compared to an ADE-model.
[5]Adjusted for atopic dermatitis.

Table 8. Incidence rate (IR) in different risk groups and incidence rate ratios (IRR) obtained in univariable and multiple Poisson regression analyses.

Variable	Cases[1] (N)	Person-years	IR per 1000 person-years (95% CI)[2]	IRR Univariable Poisson regression (95% CI)	P-value	IRR Multiple Poisson regression (95% CI)[2] (N= 3297)	P-value
Sex					0.030		0.428
Male (ref)[3]	97	13061	7.4 (6.1-9.1)	1		1	
Female	147	14782	9.9 (8.5-11.7)	1.3 (1.0-1.7)		1.1 (0.8-1.5)	
Age (years)					0.090		0.109
19-25 (ref)	19	1781	10.7 (6.8-.7)	1		1	
26-30	52	4627	11.2 (8.6-14.7)	1.0 (0.6-1.8)		1.0 (0.6-1.8)	
31-35	59	5992	9.8 (7.6-12.7)	0.9 (0.6-1.5)		0.9 (0.5-1.5)	
36-40	55	6448	8.5 (6.5-11.1)	0.8 (0.5-1.3)		0.8 (0.5-1.5)	
41-45	39	5702	6.8 (5.0-9.4)	0.6 (0.4-1.1)		0.6 (0.4-1.1)	
46-52	20	3294	6.1 (3.9-9.4)	0.6 (0.3-1.1)		0.6 (0.3-1.1)	
Zygosity					0.114		0.080
Monozygotic (ref)	115	11497	10.0 (8.3-12.0)	1		1	
Dizygotic	121	14927	8.1 (6.8-9.7)	0.8 (0.6-1.1)		0.8 (0.6-1.0)	
Atopic dermatitis[4]					<0.001		<0.001
No (ref)	184	24450	7.5 (6.5-8.7)	1		1	
Yes	59	3108	19.0 (14.7-24.5)	2.5 (1.9-3.7)		2.1 (1.6-2.8)	
Positive patch test[5]					<0.001		<0.001
No (ref)	197	26163	7.5 (6.5-8.7)	1		1	
Yes	46	1506	30.5 (22.9-40.8)	4.1 (2.9-5.6)		3.4 (2.5-4.8)	
Wet work[6]					<0.001		<0.001
No (ref)	169	21946	7.7 (6.6-9.0)	1		1	
Yes	67	4688	14.3 (11.2-18.2)	1.8 (1.4-2.5)		1.8 (1.3-2.4)	
High-risk occupation[7]					<0.001		NA

Table 8 continued

No (ref)				1	NA[8]	
Yes				1.8 (1.3-2.6)	NA[8]	
Smoking						0.660
Never (ref)	112	13647	8.2 (6.8-9.9)	1	1	
Current smoker	78	8347	9.3 (7.5-11.7)	0.9 (0.7-1.2)	1.1 (0.8-1.5)	
Ex-smoker	54	5747	9.4 (7.2-12.3)	1.0 (0.7-1.4)	1.1 (0.8-1.6)	
Alcohol						0.352
Never (ref)	37	4643	8.0 (5.8-11.0)	1	1	
≤21 drinks/week	197	21889	9.0 (7.8-10.3)	1.1 (0.8-1.6)	1.3 (0.9-1.8)	
> 21 drinks/week	9	1029	8.7 (4.6-16.9)	1.1 (0.5-2.3)	1.5 (0.7-3.3)	
Total	244	27843	8.8 (7.7-9.9)		NA[9]	NA[9]

[1]New cases of self-reported hand eczema since 1996.
[2]95 % confidence intervals.
[3]Reference group.
[4]UK Working Party's Diagnostic Criteria.
[5]Self-reported.
[6]Two hours per day of wet work/use of gloves or ≥ 20 hand washings/day.
[7] Bakers, hairdressers, dental surgery assistants, kitchen workers/cooks, butchers, health care workers, cleaners, doctors/dentists/veterinarians and laboratory technicians.
[8]Not included in the multiple regression analysis (see Materials and Methods).
[9]Not applicable.

Table 9. Drop-out analysis on study part 2 (clinical data)

Variable	Participants (%)	P-value[1]
Sex		0.532
Female	40.8%	
Male	43.3%	
Year of birth		0.009
1969-1976	35.1%	
1961-1968	41.0%	
1953-1960	50.0 %	
Zygosity		0.490
MZ	42.4%	
DZ	39.6%	
UZ	50.0%	
Previous self-reported hand eczema		0.718
Yes	41.0%	
No	42.5%	
Co-twin with previous self-reported hand eczema		0.975
Yes	41.6%	
No	41.5%	
Previous positive patch test		0.612
Yes	39.9%	
No	42.1%	
Atopic dermatitis		0.767
Yes	40.2%	
No	41.8%	

MZ: monozygotic; DZ: dizygotic; UZ: unknown zygosity.
[1]χ^2-test for comparison of two proportions. Compared with individuals declining to participate.

Table 10. Descriptive data, data on occupational consequences and number of medical consultations on individuals with hand eczema participating in clinical examination (N=188).

Sex (females/males)		68% / 32%
Age (mean years)		42 (SD 6.4)
Pairs/single twins (N)		34 / 120
Zygosity (MZ/DZ/UZ)		101 / 75 / 12
Atopic dermatitis		20.7%
Socioeconomic status	Highest/medium level	38%
	Basic level	46%
	Lowest level	15%
Wet work		30.9%
Positive patch test in 1997–98		28.2%
Clinical symptoms[1]		41.0%
HECSI score (mean)[2]		12.0 (SD 18.7)
Sick leave ever		12.4 %
	< 1 week	2.2%
	1-2 weeks	4.3%
	3-5 weeks	2.7%
	>6 weeks	3.2%
Job change ever		8.5%
Notification to the Danish National Board of Industrial Injuries		10.1%
Medical consultations	Ever	62.7%
	One visit	25.3%
	2-5 visits	22.6%
	>5 visits	15.6%

[1]Proportion of individuals with one of the following symptoms on the hands: erythema, infiltration/papules, vesicles, fissures, scaling, oedema.
[2]Mean HECSI score in individuals with visible symptoms.

Table 11. Descriptive data on twin individuals included in the analyses on allele frequencies of R501X and 2282del4.

Age (years)	41 (SD 6.6)
Sex (females/males)	65% / 35%
Hand eczema	73%
Clinical symptoms[1]	41%
Positive patch test	25%
Atopic dermatitis	14%

[1]Proportion of individuals with one of the following visible symptoms on the hands: erythema, infiltration/papules, vesicles, fissures, scaling, oedema.

13 MANUSCRIPTS I-V

See related commentary on pg 1568

Heritability of Hand Eczema Is Not Explained by Comorbidity with Atopic Dermatitis

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Genetic factors have been shown to influence the risk of hand eczema, and may theoretically influence the frequency of eruptions as well as age at onset of the disease. However, the result may be confounded by atopic dermatitis, which is a major risk factor for development of hand eczema and is known to be influenced by genetic factors. In this study, the importance of genetic and environmental risk factors in the etiology of hand eczema, independent of atopic dermatitis, was investigated in a population-based twin cohort. In addition, any possible genetic influence on frequency of hand eczema eruptions and age at onset was explored. In all, 4,128 twin individuals (response rate 82%) answered a questionnaire on self-reported hand eczema. Similarity within twin pairs was estimated and quantitative genetic modelling performed. Controlling for age and atopic dermatitis, the effect of genetic risk factors was moderate and explained 41% of the variance in liability to develop hand eczema, leaving 59% of the variance to be caused by environmental factors. Genetic factors accounted for 31% of the variance in liability regarding frequency of eruptions. Environmental factors explained the variance in liability concerning age at onset.

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INTRODUCTION

Hand eczema is characterized by a heterogeneous etiology involving both endogenous and exogenous risk factors. Atopic dermatitis is a well-established major individual risk factor for hand eczema (Rystedt, 1985). For the individual patient contact allergy constitutes an important risk factor, but the epidemiological relative risk is stronger for atopic dermatitis (Bryld *et al.*, 2003). In addition, occupational and domestic exposure to wet work increases the risk of hand eczema (Nilsson *et al.*, 1985).

Recently, a population-based twin study based on a questionnaire demonstrated that genetic effects are of significance for developing hand eczema (Bryld *et al.*, 2000). The influence of genetic effects on the liability to develop atopic dermatitis is well-known (Larsen *et al.*, 1986). Because atopic dermatitis is one of the main risk factors for hand eczema, the genetic effects on hand eczema could, at least to some extent, be explained by the coexistence of atopic dermatitis. The impact of genetic effects on contact allergy has been extensively studied. Some studies claim substantial impact of genes (Moss *et al.*, 1985), whereas newer studies find that environmental factors seem to be more important than the individual genetic background (Bryld *et al.*, 2004). Whether genetic risk factors influence the frequency of eruptions or the age at onset of hand eczema is unknown.

Twin studies are effective tools to investigate possible genetic susceptibility to a disease. By means of quantitative genetic modelling, which has become a standard statistical method, it is possible to estimate the relative importance of genetic and environmental risk factors.

The primary aim of this study was to estimate the contribution of genetic and environmental risk factors for self-reported hand eczema, while controlling for atopic dermatitis in the analysis. An additional aim was to evaluate possible genetic influence on frequency of eruptions and age at onset. Understanding the individual risk factors predisposing to hand eczema is important when planning preventive measures.

RESULTS

Response rate

A total of 4,128 twin individuals, corresponding to a response rate of 82% (4,128/5,048), answered the questionnaire. As the original cohort ascertained in 1996 comprised 6,666 twin individuals of whom 5,610 answered the 1996 questionnaire, the current responders thus comprised 74 and 62% of the twin individuals participating in 1996 and originally contacted in 1996, respectively.

Drop-out analysis

Compared to responders in 1996, there was a significantly increased proportion of female responders ($P<0.001$) and

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Abbreviations: DZ, dizygotic; MZ, monozygotic

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responders with previously self-reported hand eczema ($P<0.001$). Younger individuals were less likely to answer the questionnaire than older individuals ($P=0.004$). There was no statistically significant difference in zygosity between responders and non-responders ($P=0.242$) (Table 1).

Descriptive statistics

The responders comprised 1,717 monozygotic (MZ) twin individuals, 2,198 dizygotic (DZ) twin individuals, 167 twin individuals with unknown zygosity, 12 triplet individuals, and two quadruplet individuals. Females accounted for 59.0% of the responders. Mean age of the twin individuals was 40.4 years (SD 6.6, range 28–52). Lifetime prevalence, 1-year prevalence, and point prevalence for self-reported hand eczema was 23.7% (95% confidence interval (CI) 22.4–25.0), 11.8% (95% CI 10.8–12.8), and 5.9% (95% CI 5.2–6.6), respectively. Lifetime prevalence for atopic dermatitis was 16.4% (95% CI 15.3–17.5) and in this group 50.4 % (342/678) had self-reported hand eczema. Of those with self-reported hand eczema, 34.9% (342/979) also reported atopic dermatitis. A positive patch test was reported by 10.1% (95% CI 9.2–11.0). With regard to frequency of eruptions, 24.9% (95% CI 22.5–27.9) experienced just one episode, 54.0% (95% CI 50.5–56.7) had more than one eruption and 16.0% (95% CI 13.7–18.3) had hand eczema (nearly) all the time. The remaining 5.1% (95% CI 3.8–6.6) did not answer the question. Of the 979 twin individuals with self-reported hand eczema, 891 answered the question on calendar-year with first episode of hand eczema. The mean age at onset was 24.3 years (SD 10.5, range 0–51). Age at onset below 15 years was reported by 172 (19.3%) of the respondents with self-reported hand eczema. Approximately half (48.3%) of the respondents with age at onset below 15 years (83/172) reported atopic dermatitis.

Table 1. Drop-out analysis

Variable	Responders (%)	*P*-value[1]
Sex		<0.001
Female	2337 (84.9)	
Male	1791 (78.0)	
Previous self-reported hand eczema		<0.001
Not present	637 (77.2)	
Present	3491 (82.7)	
Year of birth		0.004
1969–1976	1241 (79.6)	
1961–1968	1593 (81.7)	
1953–1960	1294 (84.1)	
Zygosity		0.242
MZ	1717 (81.4)	
DZ	2198 (82.5)	
UZ	167 (77.0)	
Triplets	44 (78.6)	
Quadruplets	2 (100.0)	

DZ, dizygotic; MZ, monozygotic; UZ, unknown zygosity.
[1]χ^2-test for comparison of two proportions. Compared with non-responders.

Twin analyses

Self-reported hand eczema. Total number of twin pairs, cases, concordant and discordant twin pairs, lifetime prevalence and casewise concordances based on zygosity and sex, are shown in Table 2. For both sexes the concordances were higher in MZ twins than in DZ twins ($P=0.01$). The prevalence in female subjects exceeded the prevalence in male subjects ($P<0.001$).

Both unadjusted and adjusted (for age and atopic dermatitis) tetrachoric correlation coefficients are presented in Table 2. Tetrachoric correlations measure the similarity in liability to a disease between twin individuals in a twin pair. A value of zero means that there is no correlation, whereas a value of −1 or 1 reflects perfect negative or positive

Table 2. Similarity on self-reported hand eczema and total number of pairs and cases in the analyses

	Pairs (*n*)	Cases (*n*)	Concordant pairs (*n*)	Discordant pairs (*n*)	Prevalence	Casewise concordance[1]	Unadjusted tetrachoric correlation[1]	Adjusted tetrachoric correlation[1,2]
MZM	270	88	17	54	0.16	0.39 (0.25–0.52)	0.48 (0.26–0.67)	0.43 (0.18–0.63)
DZM	300	111	10	91	0.19	0.18 (0.09–0.30)	−0.01 (−0.23 to 0.22)	−0.02 (−0.26 to 0.22)
MZF	362	226	57	112	0.31	0.50 (0.42–0.58)	0.44 (0.28–0.58)	0.40 (0.24–0.55)
DZF	455	265	48	169	0.29	0.36 (0.29–0.44)	0.17 (0.01–0.32)	0.13 (−0.04 to 0.29)

MZM, monozygotic males; DZM, dizygotic males; MZF, monozygotic females; DZF, dizygotic females.
Concordant pairs denote both twins in a twin pair having self-reported hand eczema. Discordant pairs denote one twin in a twin pair having self-reported hand eczema. Casewise concordance is the risk of self-reported hand eczema in a twin individual if the co-twin has the disease. Tetrachoric correlation is the correlation in liability for self-reported hand eczema assuming an underlying latent normal distributed liability.
Cases: number of twin individuals with self-reported hand eczema.
95% confidence intervals given in parentheses.
[1]Statistically significant difference between MZM and DZM and also between MZF and DZF.
[2]Adjusted for age and atopic dermatitis.

Table 3. Results of the quantitative genetic model fitting for self-reported hand eczema, showing the relative contribution of genetic and environmental components to the total phenotypic variance (V_P)

	Genetic variance components		Environmental variance components			Comparison of nested submodels			
Model	**A**	**D**	**C**	**E**	**−2LL[1]**	**$\Delta\chi^{2}$[2]**	**Δd.f.[3]**	**AIC**	***P***
Unadjusted									
ADE	0.00 (0.00–0.48)	0.45 (0.00–0.56)	—	0.55 (0.43–0.67)	4167.14	0.54	2	4175.14	0.764
AE	0.41 (0.30–0.52)	—	—	0.59 (0.48–0.71)	4169.54	2.40	3	4175.54	0.402[4]
DE	—	**0.45 (0.33–0.57)**	—	**0.55 (0.43–0.67)**	**4167.14**	**0.54**	**3**	**4173.14**	**0.910**
E	—	—	—	1	4213.74	47.14	4	4217.74	0.000[4]
ACE	0.41 (0.22–0.52)	—	0.00 (0.00–0.14)	0.59 (0.48–0.71)	4169.54	0.07	2	4177.54	0.967
CE	—	—	0.28 (0.19–0.37)	0.72 (0.63–0.82)	4180.73	11.26	3	4186.73	0.010
Adjusted[5]									
ADE	0.00 (0.00–0.44)	0.41 (0.00–0.53)	—	0.59 (0.47–0.72)	2836.61	3.53	5	2846.61	0.618
AE	0.37 (0.24–0.49)	—	—	0.63 (0.52–0.76)	2839.13	6.05	6	2847.13	0.418[4]
DE	—	**0.41 (0.28–0.53)**	—	**0.59 (0.47–0.72)**	**2836.61**	**3.53**	**6**	**2844.61**	**0.740**
E	—	—	—	1	2870.34	37.258	7	2876.34	0.000[4]
ACE	0.37 (0.17–0.49)	—	0.00 (0.00–0.14)	0.63 (0.52–0.76)	2839.13	3.48	5	2849.13	0.627
CE	—	—	0.24 (0.14–0.33)	0.76 (0.66–0.86)	2847.98	12.33	6	2855.98	0.055

Data is fitted to different combinations of variance components in the model: $V_P=V_A+V_D+V_C+V_E$; V_A: additive genetic component, V_D: non-additive genetic component, V_C: common environmental component, V_E: unique environmental component. The model with the lowest AIC represents the model with the best fit. Selection of the best-fitting submodel is based on $\Delta\chi^2$ and an insignificant *P*-value. The best-fitting models are highlighted in bold.
95% confidence intervals given in parentheses.
[1]Minus two times log likelihood of data.
[2]Difference in χ^2 between the full model and the submodel.
[3]Difference in degrees of freedom (d.f.) between the full model and the submodel.
[4]Compared to an ADE model.
[5]Adjusted for age and atopic dermatitis.

correlation in liability, respectively. A positive correlation for female and male MZ twins was found, whereas there was no detectable correlation between DZ twins. The difference in correlation coefficients for MZ and DZ twins was statistically significant ($P=0.006$ (unadjusted) and $P=0.03$ (adjusted)). The DZ correlations were less than half of the MZ correlations, indicating the presence of dominance. Inclusion of the covariates atopic dermatitis and age led to a minor decrease in the estimates. Adjustment for atopic dermatitis had the largest effect on the correlation coefficient, whereas adjustment for age had minimal influence (data not shown).

Estimates of the different variance components from the quantitative genetic modelling (unadjusted and adjusted) as well as test statistics are given in Table 3. The DE model provided the best fit to the data both with and without inclusion of the covariates. Without adjustment genetic factors explained 45% (95% CI 33–57) of the variance in liability. The remaining 55% of the variance was attributable to unique environmental factors. With atopic dermatitis and age included in the model, the corresponding estimates were 41% (95% CI 28–53) and 59% (95% CI 47–72). With respondents reporting a positive patch test excluded from the analysis the variance components estimates only changed marginally (data not shown).

Frequency of hand eczema eruptions. Conditional probabilities and polychoric correlation coefficients, stratified by zygosity and sex, are shown in Table 4. There was a trend towards higher conditional probabilities for MZ twin pairs compared to DZ twin pairs. Increased polychoric correlation coefficients for MZ twins compared to DZ twins were seen, but this finding was statistically insignificant ($P=0.202$). The DE model provided the best fit to the data. Under this model 38% (95% CI 26–49) of the variance was attributable to genetic factors and 62% (95% CI 50–74) could be ascribed to unique environmental factors. With atopic dermatitis and age included in the model, the distribution changed to 31% (95% CI 18–43) and 69% (95% CI 57–82) (Table 5).

Age at onset. Pearson's correlations on age at onset showed an inconsistent and insignificant pattern both when all respondents were included and when only respondents above 15 years of age were included. Correlations of 0.401 ($P=0.01$) for MZ females and 0.676 ($P=0.05$) for DZ males were the only significant ones (data not shown). Only data on female twins were included in the quantitative genetic modelling analyses, because of sparse male data. The model with the best fit, with all female twins included, was the AE model. Forty-two percent (95% CI 16–61) of the variance was

Table 4. Similarity on frequency of eruptions of hand eczema

	Conditional probability				Polychoric correlation	
	Never	Only once	More than once	All the time	Unadjusted	Adjusted[1]
MZM	0.88 (0.85–0.91)	0.21 (0.00–0.40)	0.23 (0.07–0.40)	0.14 (0–0.39)	0.42 (0.19–0.61)	0.34 (0.10–0.53)
DZM	0.82 (0.78–0.86)	0.06 (0–0.16)	0.10 (0–0.21)	—[2]	−0.02 (−0.26 to 0.22)	−0.01 (−0.25 to 0.22)
MZF	0.79 (0.75–0.83)	0.23 (0.08–0.38)	0.26 (0.16–0.36)	0.14 (0.32)	0.37 (0.21–0.51)	0.30 (0.14–0.45)
DZF	0.75 (0.71–0.79)	0.09 (0–0.18)	0.19 (0.10–0.289	0.09 (0–0.21)	0.14 (−0.02 to 0.28)	0.10 (−0.06 to 0.25)

DZF, dizygotic females; DZM, dizygotic males; MZM, monozygotic males; MZF, monozygotic females.
The conditional probability gives the risk of a certain frequency of eruptions in a twin individual, given a certain frequency of eruptions in the co-twin (i.e. the risk is 0.21 that a MZM twin individual will have had hand eczema only once if the co-twin has had hand eczema only once). The polychoric correlation is a correlation in liability (in this case to frequency of eruptions) when there is more than two outcome categories.
95% confidence intervals given in parentheses.
[1]Adjusted for age and atopic dermatitis.
[2]No data, estimation impossible.

Table 5. Variance component estimates of the best-fitting model for frequency of eruptions of hand eczema, showing the relative contribution of genetic and environmental components to the total phenotypic variance (V_P)

	Genetic variance components		Environmental variance components			Comparison of nested submodels			
	A	D	C	E	−2LL[1]	$\Delta\chi^{2}$[2]	Δd.f.[3]	AIC	*P*
Unadjusted	—	0.38 (0.26–0.50)	—	0.62 (0.51–0.74)	5768.24	0.287	3	5774.24	0.963
Adjusted[4]	—	0.31 (0.18–0.43)	—	0.69 (0.57–0.82)	4031.78	2.579	6	4039.78	0.860

Selection of the models were based on AIC and secondarily on $\Delta\chi^2$ and *P*.
95% confidence intervals given in parentheses.
[1]Minus two times log likelihood of data.
[2]Difference in χ^2 between the full model and the submodel.
[3]Difference in degrees of freedom (d.f.) between the full model and the submodel.
[4]Adjusted for age and atopic dermatitis.

attributable to additive genetic factors. Using only female twins with age at onset above 15 years, the model with the best fit was an E model (data not shown), indicating that variance in unique environmental factors explained the variance in age at onset.

High-risk occupation and wet work. The concordances for high-risk occupation and wet work for MZ twins were 0.34 (95% CI 0.25–0.44) and 0.46 (95% CI 0.38–0.53), respectively. The corresponding estimates for DZ twins were 0.20 (95% CI 0.13–0.27) and 0.25 (95% CI 0.19–0.32). MZ values were statistically significantly different from DZ values ($P=0.007$ and $P=0.001$).

DISCUSSION

This study confirms the importance of genetic risk factors in the etiology of hand eczema. Forty-one percent of the variance in liability was attributable to genetic factors and the remaining variance in liability was due to unique environmental factors. The heritability was not explained by atopic dermatitis, as inclusion of atopic dermatitis in the analyses only caused a minor change in the estimates. The association between atopic dermatitis and respiratory atopy (allergic rhinitis and allergic asthma) is well-known and some studies have indicated a possible role of respiratory atopy as a risk factor for hand eczema (Lammintausta and Kalimo, 1981; Nilsson *et al.*, 1985). Whether respiratory atopy contributes to the genetic risk factors for hand eczema cannot be excluded, but was not assessed in this study.

Hand eczema has been extensively investigated in epidemiologic studies. A question similar to the one used in this study, giving the 1-year prevalence of self-reported hand eczema (''Have you had hand eczema on any occasion during the past 12 months?'') has been validated and showed relatively high specificity (96–99%), but low sensitivity (53–59%) (Meding and Barregard, 2001), and thus may underestimate the prevalence. The non-validated question on doctor-diagnosed hand eczema (''Has a doctor ever told you that you have hand eczema?'') was included to ensure agreement between the initial questionnaire in 1996 and the present questionnaire. However, all respondents answering ''yes'' to the question on doctor-diagnosed hand eczema also answered positively to the self-reported hand eczema question (data not shown).

The questionnaire-based diagnosis of hand eczema used in this study renders a definite diagnosis on the subtype of hand eczema impossible. Thus the investigated phenotype is heterogeneous and includes irritant contact dermatitis and

allergic contact dermatitis on the hands, atopic hand eczema, mixtures of the three and other types (i.e. hyperkeratotic hand eczema and pompholyx). As we knew that genetic factors significantly influence the risk of atopic dermatitis, we identified individuals with atopic dermatitis and included the variable in the model, to evaluate any change in the estimates.

The diagnosis of atopic dermatitis was based on the UK criteria, question-only version. The UK criteria are validated and widely used in epidemiologic studies (Herd *et al.*, 1996; Muto *et al.*, 2003). The validation is mostly, but not entirely, in children and for point prevalence and 1-year prevalence (Williams *et al.*, 1994b, 1996b). The prevalence of atopic dermatitis varies widely in different populations and in Northern and Western Europe estimates up to 20% have been reported (Beasley, 1998; Mortz *et al.*, 2001). Our result is in accordance with this, though considering the adult study population probably slightly overestimated. Stenberg *et al.* (2006) recently validated the question ''Have you had childhood eczema'' in an adult population and found that the question overestimated the prevalence of atopic dermatitis by a factor of 1.6. The questionnaire included two additional questions on atopic dermatitis: namely the above mentioned on self-reported atopic dermatitis and one on flexural eczema, which has previously been used in Scandinavian surveys (Susitaival *et al.*, 2003). The lifetime prevalence generated from these question were 6.7% and 12.0% (95% CI 11.0–12.9), as compared to 16.4% when using the UK criteria.

Numerous studies have evaluated possible individual genetic susceptibility to contact allergy and tried to identify genetic markers (Reich *et al.*, 2003; Westphal *et al.*, 2003). Maybe different mechanisms, and hence different genotypes may increase susceptibility to some allergens but not to others (Westphal *et al.*, 2000; Brans *et al.*, 2005). Bryld *et al.* (2004) concluded that nickel contact allergy is mainly caused by environmental exposures and only to a lesser degree by genetic factors. Unfortunately, inclusion of additional covariates in the quantitative genetic modelling was not possible owing to lack of statistical power. However, exclusion of respondents reporting a positive patch test did not change the results of the quantitative genetic modelling. This indicates that the observed effect of genetic factors was not explained by the presence of contact allergy. To our knowledge, the extent to which respondents are able to recall the results of patch testing has not been validated. The prevalence of contact allergy in this study (10.1%) is considerably lower than the 15–20% found in a previous study (Nielsen *et al.*, 2001). However, that result is based on obligatory patch testing of a population-based cohort regardless of symptoms. As expected we find a lower prevalence in this study, as only a subgroup of respondents are patch tested. Considering the lack of question validation, it is questionable whether the exclusion of respondents reporting a positive patch test, really reflects exclusion of respondents with allergic contact dermatitis on the hands.

In population-based studies and studies on occupational hand eczema the most frequent diagnosis is irritant contact dermatitis on the hands (Meding and Swanbeck, 1987; Skoet *et al.*, 2004). The diagnosis is essentially a diagnosis based on a history of exposure to irritants and lack of positive patch tests. Little is known about possible individual genetic susceptibility to irritant contact dermatitis, other than atopic dermatitis. A few studies indicate that genetic factors are of importance. Allen *et al.* (2000) identified a non-atopic genetic marker for irritant contact dermatitis in normal individuals. Bryld *et al.* (2003) when analyzing data from a population-based clinical twin study, found evidence that hitherto unknown genetic risk factors influence the risk of irritant contact dermatitis on the hands. Considering our attempt to control for atopic dermatitis and contact allergy in the analyses, and the usually high proportion with irritant hand eczema, one can argue that the genetic risk factors found in this study primarily relate to the risk of acquiring irritant contact dermatitis on the hands.

Hand eczema occurs twice as often in women compared to men. This difference is generally ascribed to differences in environmental exposures, rather than to sex difference in skin susceptibility (Agner and Menné, 2006). Most experimental studies have found no sex difference in skin reactivity to irritants (Bjornberg, 1975). Regarding sex difference and skin susceptibility to contact allergens, the present knowledge is limited and inconclusive. We found no evidence of sex difference regarding the distribution of genetic and environmental risk factors for hand eczema, thus supporting previous knowledge.

Genetic factors accounted for 31% of the variance in liability regarding frequency of eruptions.

Genetic modelling of the data resulted in variance components comparable to the estimates for hand eczema, but with a slightly higher influence from environmental factors, especially with the covariates included in the model. Environmental factors explained almost 70% of the variance in liability concerning frequency of eruptions. The result underlines the importance of treatment and secondary preventive measures. Finally the quantitative genetic modelling procedure was applied to the data on age at onset. The calculation was hampered by a lack of data, as only concordant twin pairs with respect to hand eczema could be included in the analysis. The best-fitting models were an AE model and an E model, the last one indicating that unique environmental factors determine age at onset.

To extrapolate the results to the general population, the twin population must be fairly representative of the background population. The study population is drawn from the twin cohorts in the Danish Twin Registry, which is based on the Danish Civil Registration System and covers 74.4% of all twins born 1953–1967 (inclusive) and 97.4% of those born 1968–1982 (inclusive) (Kyvik *et al.*, 1995). The study population included all twins born 1953–1976 living on the island of Sealand or its neighboring islands. The responders in this study comprise 62% of the twin individuals originally contacted in 1996 and 74% of the twin individuals responding in 1996. As both questionnaires have focused on hand eczema, a selection in favor of participants with (hand) eczema problems, may have occurred, possibly

resulting in an overestimation of the prevalence of hand eczema and the risk estimates. Drop-out analysis in this study demonstrated a predominance of female twin individuals and twin individuals with previous self-reported hand eczema among the responders, which could also increase the prevalence estimate. However, the prevalence of hand eczema and atopic dermatitis in this study are comparable to previous estimates from non-twin population-based studies.

An important assumption in the classical twin study is the one of equal environment for the two types of twins. If however, the MZ twins are treated more similar than the DZ twins, a greater phenotypic similarity of the MZ twins is not only owing to a greater genetic similarity, but also to a greater environmental similarity and thus the effect of genetic factors will be overestimated. Wet work and certain occupations increase the risk of hand eczema (Skoet *et al.*, 2004). By calculating concordances for high-risk occupations and wet work we wanted to assess the similarity in exposure for MZ and DZ twins. One could speculate that MZ twins will choose more similar occupations and thus have a more similar exposure than DZ twins. We found statistically significantly higher concordances for high-risk occupation and exposure for MZ than for DZ twins and this may potentially have inflated the estimate of the genetic variance components. However, studies have shown that it is very difficult to obtain reliable exposure data (Jungbauer *et al.*, 2004) and job titles are not a very good proxy for actual exposure (Anveden *et al.*, 2006).

The effect of epigenetic modifications (DNA changes arising after separation of the embryonic cells, for example DNA methylation) may undermine the important assumption of identical genes in MZ twins and hence phenotypic discordance between MZ twins cannot solely be ascribed to differences in environmental influence (Singh *et al.*, 2002; Fraga *et al.*, 2005). The frequency and importance of epigenetic factors in twin studies are at present poorly understood.

The biometric model assumes absence of significant gene–environment interaction and correlation. Gene–environment interaction relates to the way genes and environment affect the phenotype, that is, the same environmental influence has differential effects on different genotypes. Finally, the model assumes random mating. Assortative mating, which is nonrandom mating based on other factors than biological relatedness, tend to increase the genetic and environmental similarity between relatives. In a twin study, assortative mating would increase the similarity between DZ twins relative to MZ twins and if so, underestimate the importance of genetic factors. To what extent these last three assumptions are satisfied in this study is unknown.

The best-fitting model in the quantitative genetic modelling for hand eczema was a DE model. As dominance (D) is non-additive interaction of alleles at the same locus, dominance variance (V_D) only contributes to the genetic covariance between full siblings (including MZ and DZ twins) and not to the genetic covariance between other relatives. Additive variance (V_A) is thus the chief cause of resemblance between relatives and the DE model is biologically very unlikely (Falconer and Mackay, 1996a, b). The limited sample size and a resulting lack of statistical power, is a possible explanation for the best-fitting model in this study being a DE model. In a larger cohort we might have found that the ADE model had the best fit.

The basis of quantitative genetics is the fact that variations in DNA give different phenotypes. Quantitative genetics allow us to estimate the importance of genetic risk factors; unfortunately, it does not allow us to characterize the involved genes.

In conclusion, this study showed that genetic factors independent of atopic dermatitis are of moderate etiological importance for hand eczema and frequency of hand eczema eruptions. No effect of genetic factors on age at onset was found. The remaining culprit is to further characterize and pinpoint the complex phenotype(s) and subtypes of hand eczema, where genetic factors have the largest influence. Preventive measures, both primary and secondary, are of unchanged importance.

MATERIALS AND METHODS

Study design and population

The present study is a follow-up study on a twin cohort ascertained from the population-based Danish Twin Registry (Bryld *et al.*, 2000).

Previous study. In autumn 1996, a mailed questionnaire was answered by 5,610 twin individuals born between 1953 and 1976 (response rate 84%). Both members of the twin pair had to reside on Sealand or one of the neighboring islands at the time of ascertainment. The cohort comprised MZ and DZ twin pairs, a few triplets and quadruplets as well as a minor group with unknown zygosity. Zygosity was determined in a previous questionnaire study based on the similarity method, which is previously described (Kyvik *et al.*, 1995). This method determines correct zygosity in more than 95% of cases (Christiansen *et al.*, 2003). Because of expected differences in hand eczema prevalence for female and male twins, only same-sex twin pairs were included.

Present study. In January 2005 a new questionnaire was sent by mail to the previously participating twin individuals. Addresses were obtained from the Danish Civil Registration System on 5,048 twin individuals. The remaining twins had a protected address, had emigrated, were dead or impossible to trace. A return envelope was enclosed with the questionnaire and one reminder was mailed to non-responders after 1 month. The Danish Act on Scientific-Ethical Committees and Biomedical Research does not require approval by a Scientific-Ethical Committee for questionnaire surveys and thus, this was not applied for.

Questionnaires

If a twin individual reported hand eczema in 1996, this individual was regarded as having the phenotype and obtained a different questionnaire than those twin individuals without self-reported hand eczema in 1996. Only twin individuals without self-reported hand eczema in 1996, where asked about self-reported hand eczema in the new questionnaire. Altogether 4,223 questionnaires were mailed

to twin individuals without previously self-reported hand eczema and 825 questionnaires were mailed to twin individuals with previously self-reported hand eczema. A group of 14 twin individuals reporting hand eczema in 1996 made a marginal note in the questionnaire denying previous hand eczema and in the data analyses they were merged with the group without self-reported hand eczema.

The questionnaires additionally included questions on atopic dermatitis (UK criteria, question-only version (Williams *et al.*, 1994a; Williams, 1996a)) and questions on positive patch tests to nickel or other contact allergens (preservatives, perfume, rubber, plants, chromate, or others). Frequency of eruptions was investigated with questions from The Nordic Occupational Skin Questionnaire (NOSQ-2002) (question number D4) (Susitaival *et al.*, 2003). Respondents without self-reported hand eczema bypassed the questions on frequency of eruptions. Skin exposure was assessed indirectly with a question on job titles and directly with questions on hours of wet work per day, hours with glove use per day and number of hand washings per day. The questions on direct skin exposure were adapted from the NOSQ-2002 (question numbers E1, E2, E5, and E8).

Definitions

Self-reported hand eczema. A diagnosis of self-reported hand eczema was defined as ''yes'' to one of the questions ''Have you ever had hand eczema?'' or ''Has a doctor ever told you that you have hand eczema?'' *Frequency of eruptions:* Respondents were divided into four groups, having had hand eczema (1) never, (2) only once, (3) more than once, and (4) (nearly) all the time.

Age at onset. The calendar-year of first episode of hand eczema was subtracted from birth-year to obtain the age at onset.

Atopic dermatitis. This was defined using the UK-criteria, question-only version.

Contact allergy. A positive answer to a question on positive patch tests was considered indicative of contact allergy.

High-risk occupation. High-risk occupation was defined as employment in one of nine occupations, previously identified as high-risk occupations for hand eczema (bakers, hairdressers, dental surgery assistants, kitchen workers/cooks, butchers, health care workers, cleaners, doctors/dentists/veterinarians, and laboratory technicians) (Skoet *et al.*, 2004).

Wet work. Wet work was defined as skin exposed to liquids more than 2 hours per day, or use of occlusive gloves more than 2 hours per day, or very frequent hand washing (>20 times/day) (Diepgen and Coenraads, 1999).

Statistics

Descriptive statistics. In the descriptive analyses all twin individuals (MZ, DZ, unknown zygosity, triplets, and quadruplets) were used. Data on both single twin individuals and complete twin pairs were used when calculating means and prevalences. Data were analyzed using SPSS version 13.0. The χ^2 test for comparison of two proportions was used in the drop-out analysis.

Twin analyses. The classical twin study relies on the assumption that MZ twins have identical segregating genes. If then, any difference (discordance) for a disease is present this is attributed to environmental influence. DZ twins share on average 50% of their genes and any difference is therefore owing to a combination of genetic and environmental factors. If genetic factors have any substantial influence on disease liability, then a greater phenotypic similarity between MZ twin individuals, than between DZ twin individuals is expected. Twin similarity was assessed by means of casewise concordances, conditional probabilities, and correlation coefficients, stratified by zygosity and sex. This was followed by quantitative genetic modelling to estimate the relative importance of genetic and environmental factors. Casewise concordances were estimated using Stata Statistical Software. Conditional probabilities for variables with more than two outcomes were estimated using Maple version 10. Estimation of tetra- and polychoric correlation coefficients and quantitative genetic modelling were performed with the software package Mx (Neale *et al.*, 2003). Pearson correlations were calculated with SPSS version 13.0. Only data on complete MZ and DZ twin pairs were used in the twin analyses.

Casewise concordances and conditional probabilities. The casewise concordance is a conditional probability and gives the probability for disease in a twin individual, if the co-twin is affected (Kyvik, 1997). If the MZ casewise concordance exceeds the DZ casewise concordance, it indicates an effect of genetic factors on disease expression. Because of expected complete and independent ascertainment of twin individuals we used the casewise concordance formula: $CR = 2C/(2C + D)$; where C is the number of concordant pairs and D is the number of discordant pairs. The 95% CIs were calculated using bootstrap methods (Efron and Tibshirani, 1986). Equality between MZ and DZ concordances was tested in a one-sided test. Casewise concordances were calculated for self-reported hand eczema. Similarity in frequency of eruptions was assessed with conditional probabilities for variables with more than two outcomes.

To investigate the correctness of the equal environment assumption, casewise concordances for high-risk occupations and wet work were estimated.

Correlations. The correlation in disease liability within twin pairs was expressed as tetrachoric correlation coefficients (dichotomous outcome i.e. self-reported hand eczema) or polychoric correlation coefficients (more than two categories i.e. frequency of eruptions). Determination of tetrachoric correlation coefficients relies on the assumption of a normal-distributed underlying liability (susceptibility) to develop a disease. The disease is expressed, when the individual exceeds a certain threshold on the liability distribution.

Age and atopic dermatitis were included in the model assuming a linear effect on the thresholds by the covariates. Age was chosen as a covariate as it is previously shown that hand eczema is more prevalent in younger persons. Owing to the limited size of the study cohort, it was not possible to stratify the material into different age groups. The second covariate, atopic dermatitis, was predetermined owing to the aim of the study. The difference in tetra- and polychoric correlation coefficients between MZ and DZ twins was assessed with a likelihood ratio test (Neale and Cardon, 1992).

Correlations in age at onset (continuous variable) were assessed with Pearson correlation coefficients.

Quantitative genetic modelling. Phenotypic variation may be attributed to genetic and environmental causes. In quantitative genetic modelling, which is widely used with twin data, the total phenotypic variance is decomposed into four variance components: Additive genetic variance (V_A), non-additive genetic variance (V_D), shared/common environmental variance (V_C), and unique (individual-specific) environmental variance (V_E). A represents the influence of alleles at several loci acting in an additive manner (i.e. with the same weight). D represents the presence of dominance, which is non-additive interaction of alleles at the same locus. *C* reflects environmental influence shared by members of a twin pair and *E* reflects environmental influence unique to the individual. Heritability in the broad sense is defined as the proportion of the phenotypic variance that is attributable to genetic variance ($V_A + V_D$). MZ twin pairs are perfectly correlated for genetic variance ($V_A + V_D$), whereas the genetic difference between DZ twin pairs corresponds to a correlation of 0.5 for additive genetic variance and 0.25 for dominant genetic variance. By definition (equal environment assumption) common environment (V_C) is perfectly correlated between both MZ and DZ twin pairs. Correlation in unique environment (V_E) is zero in both zygosity groups. Based on this, the equations for the expected variances and twin covariances (or correlations) are as follows:

$$V_P = V_A + V_D + V_C + V_E$$
$$COV_{MZ} = V_A + V_D + V_C$$
$$COV_{DZ} = 0.5\,V_A + 0.25\,V_D + V_C$$

V_P is the total phenotypic variance, COV_{MZ} is the covariance within MZ twin pairs and COV_{DZ} is the covariance within DZ twin pairs.

The modelling procedure tests these expected variance–covariance matrices against the observed data pattern, and the aim is to explain the pattern with the use of as few parameters as possible. The model assumes random mating, no effect of epigenetic factors, no gene–environment interaction or correlation and no epistasis (a particular allele interacts with alleles at other loci). V_D and V_C are confounded in a twin study with MZ and DZ twins reared together, and cannot be discriminated in the same model. The presence of D tends to produce DZ correlations less than half the size of the MZ correlations. Influence of C is indicated by a DZ correlation above 50% of the MZ correlation.

First, full models (ADE and ACE) were examined, stratified by sex, and secondly a model assuming equal standardized variance components for both sexes was attempted. Equal variance components across sex were confirmed and then nested (i.e. one model is a restricted version of the other) submodels (AE, DE and E and AE, CE and E, respectively) were computed on a data set comprising both sexes. Same procedure was followed with the covariates, atopic dermatitis and age, included in the model.

It was not possible to include further covariates in the model, owing to lack of statistical power. Instead, in a new calculation, all twin individuals reporting a positive patch test were excluded. This was considered an indirect measure of the influence of contact allergy on the variance estimates. Excluding respondents with a positive patch test, was restricted to the quantitative genetic modelling for self-reported hand eczema.

Selection of the best-fitting model is based on Akaike Information Criterion (AIC) (Akaike, 1987; Neale and Cardon, 1992). This is defined as $-2LL + 2q$, where $-2LL$ is minus twice log likelihood of data and q is the number of free parameters in the model. The criteria takes into account how well the data fits the model and the degree of parsimony (i.e. increase in the number of parameters in the model is stopped, if this does not lead to a significantly better fit to the data) of the model. The model with the lowest AIC represents the model with the best fit to the data and the most parsimonious model. Nested submodels were compared to the full models using the χ^2 test statistic. A high χ^2 and an insignificant *P*-value indicate that the model offers a good description of the data.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Incidence of hand eczema in a population-based twin cohort: genetic and environmental risk factors

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Conflicts of interest
None declared.

Summary

Background Population-based studies on the incidence of hand eczema are sparse.
Objectives The aim of this prospective follow-up study was to determine the incidence rate of hand eczema in a population-based twin cohort. Secondly, the role of genetic factors and other potential risk factors for hand eczema was investigated.
Methods A questionnaire on self-reported hand eczema was answered by 5610 and 4128 twin individuals in 1996 and 2005, respectively. Data were analysed in a Poisson regression analysis.
Results The crude incidence rate was 8·8 cases per 1000 person-years (95% confidence interval, [CI] 7·7–9·9). Incidence rate ratios (IRRs) dependent on the co-twin's hand eczema status revealed a significant, doubled risk for monozygotic twin individuals with a co-twin affected by hand eczema, compared with dizygotic twin individuals with a co-twin affected by hand eczema (IRR 2·4, 95% CI 1·4–4·1). Also, significantly increased IRRs were found for positive patch test, atopic dermatitis, and wet work.
Conclusions Hand eczema is still a frequent disease and genetic factors are confirmed important risk factors. Positive patch test, atopic dermatitis and wet work were associated with an increased risk, whereas no association with age, sex, smoking or alcohol was found.

Epidemiological studies on the occurrence of hand eczema are numerous. The most frequent design is cross-sectional, with the measure of disease frequency expressed as a point, 1-year or lifetime prevalence. Some studies are population-based[1–4] whereas others focus on highly selected, often high-risk populations such as dentists, car mechanics or hairdressers.[5–9]

The number of studies on incidence, particularly prospective population-based studies, is limited. Very high estimates have been found in high-risk groups such as nurses and hairdressing apprentices (145–328 cases per 1000 person-years).[8,10] Recently, Lind *et al.*[9] reported an incidence rate of 23·8 cases per 1000 person-years in hairdressers. In other groups (i.e. office and car industry apprentices) incidence rates of 41 and 47 cases per 1000 person-years, respectively, have been reported.[11,12] Retrospective studies on the incidence rate of hand eczema in the general population have found estimates ranging from 4·4 to 7·9 cases per 1000 person-years.[13–15]

Twin studies can be used to reveal a possible genetic influence on the aetiology of diseases by comparing the occurrence of disease in monozygotic (MZ) and dizygotic (DZ) twins. It has previously been shown that genetic factors independent of atopic dermatitis are of importance for the development of hand eczema.[16,17]

The relation between tobacco smoking and the risk of hand eczema is uncertain. Only a few studies have evaluated the subject, and the results are conflicting.[18–20] The question as to whether alcohol intake is a risk factor for hand eczema has, to our knowledge, not yet been scientifically evaluated.

The aim of this study was to determine the incidence rate of hand eczema in a prospectively followed population-based twin cohort. In addition, any possible influence of the co-twin's hand eczema status, and of smoking and alcohol intake, as well as factors previously shown to increase the incidence rate of hand eczema, such as sex, age, atopic dermatitis, positive patch test, wet work and high-risk occupation, were evaluated.

Materials and methods

Study design and population

This study was a follow-up study on a previous questionnaire survey. In autumn 1996 all same-sex twin pairs (in total 6666 twin individuals) living on Zealand or one of the

neighbouring islands and born between 1953 and 1976 received a short questionnaire with 10 questions on self-reported hand eczema and symptoms of hand eczema. A total of 5610 twin individuals answered the questionnaire (response rate 84%). Results from this study have previously been published.[16]

In January 2005 a second questionnaire was distributed to the previously participating twin individuals who were alive and resident in Denmark (5048 twin individuals). Individuals with unavailable addresses had a protected address, had emigrated, were dead or were impossible to trace. The cohort included MZ and DZ twin individuals, a few triplets and quadruplets, as well as a minor group with unknown zygosity. Zygosity was determined in a previous questionnaire study and was based on the similarity method.[21] It has previously been shown that this method determines correct zygosity in more than 95% of cases.[22] As the Danish Act on Scientific-Ethical Committees and Biomedical Research does not require approval by a Scientific-Ethical Committee for questionnaire surveys this was not applied for.

Questionnaires

Two different questionnaires were distributed in 2005 depending on whether the individual reported hand eczema in 1996 or not. Only individuals without self-reported hand eczema in 1996 were asked about self-reported hand eczema in the new questionnaire. The remaining questions were identical in the two questionnaires. Altogether 825 questionnaires were mailed to individuals with self-reported hand eczema in 1996 and 4223 questionnaires were mailed to individuals without self-reported hand eczema in 1996. A group of 14 individuals reporting hand eczema in 1996 denied previous hand eczema by making a note in the questionnaire on return. In the data analyses they were merged with the group without self-reported hand eczema. Only individuals without previous self-reported hand eczema (and the 14 individuals denying previous hand eczema) were included in the data analyses.

The questionnaires additionally included questions on atopic dermatitis, positive patch tests, skin exposure, job titles, smoking habits and alcohol consumption. Individuals with self-reported hand eczema were asked about the year of onset.

Definitions

Self-reported hand eczema was defined as 'yes' to the question 'Have you ever had hand eczema?' The U.K. criteria, question-only version, were used to diagnose *atopic dermatitis*.[23,24] A *positive patch test* was defined as a positive answer to a question on previous positive patch tests (to nickel, preservatives, perfume, rubber, chromate or other). *Wet work* was defined as skin exposed to liquids for > 2 h per day, or use of occlusive gloves for > 2 h per day, or very frequent hand washing (> 20 times per day).[25] The questions on skin exposure to liquids and gloves and on number of hand washings were adapted from the NOSQ-2002.[26] *High-risk occupation* was defined as employment in one of nine occupations previously identified as high-risk occupations for hand eczema, namely bakers, hairdressers, dental surgery assistants, kitchen workers/cooks, butchers, healthcare workers, cleaners, doctors/dentists/veterinarians and laboratory technicians.[27] Regarding *smoking and alcohol*, based on smoking history individuals were primarily stratified into three separate categories: (i) never smokers; (ii) current smokers; and (iii) ex-smokers and secondarily on the basis of pack-years (1 pack-year = 15 cigarettes per day for 1 year) into the following categories: (i) 0 pack-years; (ii) ≤ 15 pack-years; and (iii) > 15 pack-years. Based on open questions on mean weekly intake of beer (bottles), wine (glasses) and spirits (units), individuals were divided into three categories depending on alcohol intake: (i) never; (ii) ≤ 21 drinks per week; and (iii) > 21 drinks per week. One drink was defined as one bottle of beer, one glass of wine or one unit of spirits.

Statistical analyses

The χ^2 test for comparison of two proportions was used in the drop-out analysis. An individual contributed with 8·5 person-years at risk if the individual did not develop hand eczema (from 1 October 1996 to 31 March 2005). Calculation of person-years for individuals who developed hand eczema was based on the year of onset of hand eczema. As 27 individuals did not report the year of onset they were assigned the year 2000 (middle of study period) as the year of onset. Incident cases were assumed to develop hand eczema in the middle of the year of onset (i.e. an individual reporting year of onset in 2000 contributed with 3·75 person-years at risk). The crude incidence rates as well as incidence rates dependent on sex, zygosity, age, atopic dermatitis, positive patch test, wet work, high-risk occupation, smoking and alcohol were determined (all twin individuals with no missing values included). By taking account of changing age during the study period, a twin individual contributed with person-years in more than one age-group.

The effect of risk factors on incidence of hand eczema was evaluated with Poisson regression using the command 'poisson' in Stata (StataCorp, College Station, TX, U.S.A.). Effects are presented as incidence rate ratios (IRRs) with 95% confidence intervals (CIs). The CIs are corrected for intra-twin correlation using the option 'cluster' in Stata.

The effect of sex, age, atopic dermatitis, positive patch test, wet work, high-risk occupation, smoking and alcohol was evaluated in univariable Poisson regression analyses. A total of 759 respondents failed to answer the question on job title. Therefore, to avoid losing too many data, the variable *high-risk occupation* was excluded from the final multiple Poisson analysis. Also, there was a considerable overlap between the variables *high-risk occupation* and *wet work*. Almost 90% answered either yes or no to both wet work and high-risk occupation. Due to the predetermined aim of the study, all other covariables were included in the multiple Poisson regression model, regardless of statistical significance in the univariable model. Respondents

with missing values in any one of the variables were excluded from the multiple Poisson regression model, leaving 3297 for the analysis. Test for interaction was performed for sex and atopic dermatitis against all other variables and for a few other selected combinations.

The incidence rate for twin individuals with and without a co-twin affected by hand eczema was determined separately for MZ and DZ twin individuals. Information on the co-twin's hand eczema status was available in only 2886 twin individuals (out of 3221 MZ and DZ twin individuals), restricting the analysis to this group. As year of onset was known, the co-twin's hand eczema status could change during the follow-up period (i.e. if the co-twin became an incident case). Thus the total number of twin individuals at risk exceeds the number available for analysis (2886), as a twin individual could contribute with time at risk in both the analysis of twin individuals with unaffected co-twins and the analysis of twin individuals with affected co-twins. An IRR estimating the risk of hand eczema in MZ as opposed to DZ twin individuals with and without a co-twin affected by hand eczema was determined and adjusted for any effect of sex, age, atopic dermatitis, positive patch test and wet work.

SPSS version 13.0 (SPSS, Chicago, IL, U.S.A.) was used for data management and drop-out analyses. Additional analyses were performed with Stata statistical software. All P-values are two sided and a 5% significance level was used.

Results

In the drop-out analysis there was a significantly increased proportion of female responders ($P < 0{\cdot}001$) in 2005, and younger individuals were less likely to answer the questionnaire than older individuals ($P = 0{\cdot}004$) (data not shown).

After one reminder, 4128 twin individuals answered the questionnaire (response rate 82%). Of these, 623 twin individuals already had self-reported hand eczema in 1996 and are thus not part of the population at risk. A total of 356 twin individuals without self-reported hand eczema in 1996

Table 1 Incidence rate (IR) in different risk groups and incidence rate ratio (IRR) obtained in a multiple Poisson regression analysis with all variables included

Variable	Cases[a] (n)	Person-years	IR per 1000 person-years (95% CI)[b]	IRR (95% CI)[b] (n = 3297)	P-value
Sex					
Male (ref)[c]	97	13 061	7·4 (6·1–9·1)	1	0·428
Female	147	14 782	9·9 (8·5–11·7)	1·1 (0·8–1·5)	
Age (years)					
19–25 (ref)	19	1781	10·7 (6·8–16·7)	1	0·109
26–30	52	4627	11·2 (8·6–14·7)	1·0 (0·6–1·8)	
31–35	59	5992	9·8 (7·6–12·7)	0·9 (0·5–1·5)	
36–40	55	6448	8·5 (6·5–11·1)	0·8 (0·5–1·5)	
41–45	39	5702	6·8 (5·0–9·4)	0·6 (0·4–1·1)	
46–52	20	3294	6·1 (3·9–9·4)	0·6 (0·3–1·1)	
Zygosity					
Monozygotic (ref)	115	11 497	10·0 (8·3–12·0)	1	0·080
Dizygotic	121	14 927	8·1 (6·8–9·7)	0·8 (0·6–1·0)	
Atopic dermatitis[d]					
No (ref)	184	24 450	7·5 (6·5–8·7)	1	< 0·001
Yes	59	3108	19·0 (14·7–24·5)	2·1 (1·6–2·8)	
Positive patch test[e]					
No (ref)	197	26 163	7·5 (6·5–8·7)	1	< 0·001
Yes	46	1506	30·5 (22·9–40·8)	3·4 (2·5–4·8)	
Wet work[f]					
No (ref)	169	21 946	7·7 (6·6–9·0)	1	< 0·001
Yes	67	4688	14·3 (11·2–18·2)	1·8 (1·3–2·4)	
Smoking					
Never (ref)	112	13 647	8·2 (6·8–9·9)	1	0·660
Current smoker	78	8347	9·3 (7·5–11·7)	1·1 (0·8–1·5)	
Ex-smoker	54	5747	9·4 (7·2–12·3)	1·1 (0·8–1·6)	
Alcohol					
Never (ref)	37	4643	8·0 (5·8–11·0)	1	0·352
≤ 21 drinks per week	197	21 889	9·0 (7·8–10·3)	1·3 (0·9–1·8)	
> 21 drinks per week	9	1029	8·7 (4·6–16·9)	1·5 (0·7–3·3)	
Total	244	27 843	8·8 (7·7–9·9)	NA[g]	NA[g]

[a]New cases of self-reported hand eczema since 1996; [b]95% confidence intervals; [c]reference group; [d]U.K. Working Party criteria; [e]self-reported; [f]2 h per day of wet work/use of gloves or ≥ 20 hand washings per day; [g]not applicable.

Table 2 Incidence rate (IR) per 1000 person-years dependent on hand eczema status of co-twin, sex and zygosity and incidence rate ratio (IRR) adjusted for sex, age, atopic dermatitis, positive patch test and wet work

	Twin individuals at risk (n)	Cases[a] (n)	Person-years	IR per 1000 person-years (95% CI)[b]	IRR (MZ/DZ) (95% CI)[b,c]
Co-twins *with* hand eczema					
MZM	80	12	559	21·5 (12·2–37·8)	
MZF	150	21	1043	20·1 (13·1–30·9)	
Total MZ	230	33	1602	20·6 (14·6–29·0)	2·4 (1·4–4·1)
DZM	135	6	1033	5·8 (2·6–12·9)	
DZF	230	20	1673	12·0 (7·7–18·5)	
Total DZ	365	26	2706	9·6 (6·5–14·1)	
Co-twins *without* hand eczema					
MZM	544	29	4444	6·5 (4·5–9·4)	
MZF	566	40	4506	8·9 (6·5–12·1)	
Total MZ	1110	69	8950	7·7 (6·1–9·8)	1·0 (0·7–1·4)
DZM	598	33	4820	6·8 (4·9–9·6)	
DZF	698	46	5649	8·1 (6·1–10·9)	
Total DZ	1296	79	10 469	7·5 (6·1–9·4)	

MZ, monozygotic; DZ, dizygotic; MZM, monozygotic male; MZF, monozygotic female; DZM, dizygotic male; DZF, dizygotic female. [a]New cases of self-reported hand eczema since 1996; [b]95% confidence intervals; [c]IRR for MZ twins compared with DZ twins in co-twins *with* hand eczema is statistically significantly different from IRR for MZ twins compared with DZ twins in co-twins *without* hand eczema ($P = 0·007$).

reported present or previous eczema in the questionnaire in 2005. However, a subgroup of these, in total 112 twin individuals, reported onset of hand eczema before 1996 and was thus regarded neither as incident cases nor as part of population at risk. The resulting number of new cases was thus 244 and the total number of twin individuals without previous self-reported hand eczema in 1996 was 3393 (statistical analyses based on this sample). The total number of person-years under observation was 27 843. This yielded a crude incidence rate of 8·8 cases per 1000 person-years (95% CI 7·7–9·9).

Incidence rates dependent on sex, age, zygosity, atopic dermatitis, positive patch test, wet work, smoking and alcohol are shown in Table 1. Incidence rates for triplets, quadruplets and those of unknown zygosity are not shown due to sparse data.

Smoking, alcohol, zygosity and age were statistically insignificant in the univariable Poisson regression analysis (data not shown). Both when looking at never smokers/current smokers/ex-smokers and at pack-years there was no risk difference between the groups. Female sex, atopic dermatitis and positive patch test were associated with an increased risk. Individuals reporting wet work or being employed in a high-risk occupation had an almost doubled IRR. In the multiple Poisson regression (Table 1) only atopic dermatitis, positive patch test and wet work were significant predictors for hand eczema. No statistically significant interaction between variables was detected.

The incidence rate in MZ twin individuals with co-twins having hand eczema was 20·6 (95% CI 14·6–29·0) cases per 1000 person-years, whereas in comparable DZ twin individuals the incidence rate was 9·6 (95% CI 6·5–14·1) cases per 1000 person-years. In twin individuals with co-twins *not* having hand eczema the incidence rates in MZ and DZ twin individuals were comparable (Table 2). MZ twin individuals having a co-twin with hand eczema had an increased risk of hand eczema compared with DZ twin individuals with an affected co-twin (IRR 2·4, 95% CI 1·4–4·1) adjusted for sex, age, atopic dermatitis, positive patch test and wet work. This result was statistically significantly different ($P = 0·007$) from the IRR determined for MZ compared with DZ twin individuals with nonaffected co-twins (IRR 1·0, 95% CI 0·7–1·4) (Table 2).

Discussion

The crude incidence rate of hand eczema found in this prospective study is comparable with, although slightly higher than, estimates from previous retrospective population-based studies.[13–15] Presence of hand eczema was determined by questionnaire, using a question very similar to a thoroughly validated question ('Have you had hand eczema on any occasion during the past 12 months?'), which has proven to have a high specificity (96–99%), but less sensitivity (53–59%).[28] Use of this question would thus tend to decrease rather than increase the incidence rate. There was a predominance of female responders, which could inflate the incidence rate.

An interesting finding in this study was the demonstration of a more than doubled risk in MZ twin individuals with co-twins having hand eczema compared with DZ twin individuals with the same status. This confirms the importance of genetic risk factors in the pathogenesis of hand eczema. Genetic factors are important predictors for atopic dermatitis, whereas the role of genetic factors in the aetiology for contact allergy

is debated and, if present, is considered of less importance. The analysis was adjusted for atopic dermatitis and positive patch test, and thus the effect of genetic factors was not explained by these factors.

It is well known that the prevalence of hand eczema among females exceeds the prevalence in males. This has been ascribed to differences in exposure, not to sex differences in skin reactivity to irritants and/or allergens. Female sex was a risk factor in the univariable analysis, but the effect disappeared in the multiple analysis. Subanalyses showed that inclusion of the covariate wet work was responsible for the elimination, thus confirming that skin exposure and not female sex itself increases the risk of acquiring hand eczema. Also, Meding and Jarvholm[15] found equal incidence rates in women and men above 30 years of age. A possible explanation for the higher occurrence in young females is their frequent simultaneous domestic (i.e. household and caring for young children) and occupational exposure to irritants.

The strongest risk factors for hand eczema in this study were positive patch test and atopic dermatitis, which is in line with previously published work. Positive patch test is seemingly the most important risk factor. Data may, however, be biased. Information bias is likely, as respondents who have acquired hand eczema probably will tend to have a patch test performed more often than respondents without hand eczema. Also, respondents with hand eczema may be able to recall their (positive) test results to a higher degree than respondents without hand eczema. These possible biases may inflate the risk estimate. On the other hand, one can argue that individuals with a positive patch test will be more careful and observant with respect to skin changes and exposure, thus diminishing this source of bias. This is supported by a study with 20 years of follow-up, where it was found that positive patch test to nickel in childhood did not increase the risk of hand eczema later in life.[29] As expected, the incidence rates in MZ and DZ twin individuals (all twin individuals included regardless of co-twins' hand eczema status) were not statistically significantly different. This was expected as being either an MZ or a DZ twin does not increase the risk of hand eczema.

The debated association between smoking and risk of hand eczema was thoroughly investigated in this large cohort. Edman[20] found that smoking (variable not defined) was a risk factor for vesicular palmar eczema in males and Montnemery *et al.*[19] found that smoking (more than five cigarettes per day) was an independent risk factor for reporting 1-year prevalence of hand eczema (OR 1·35, 95% CI 1·04–1·75). In another study, in metalworker trainees, there was no significant difference in the number of cigarettes smoked per day in the group with hand eczema compared with a group without hand eczema.[18] Recently, Linneberg *et al.* reported that smoking (> 15 pack-years) was significantly associated with contact allergy.[30] This has yet to be confirmed and any possible implications for the association between smoking and hand eczema are unknown. Our data did not show any increased risk depending on smoking status, nor any association between pack-years and hand eczema.

The clinical impression that alcohol can be an aetiological factor for hand eczema could not be confirmed in this study. Possibly, alcohol is only of importance in a few selected individuals with an actual misuse of alcoholic beverages. The number of such cases in a population-based cohort study will be too small to demonstrate any possible increased risk. However, in the majority of cases alcohol consumption seems to be of no importance with respect to risk of hand eczema.

The prospective determination of incident cases since 1996 in this study is methodologically valuable. However, the explanatory variables (except for the co-twin's hand eczema status, sex, age and zygosity) were determined in 2005 and thus conclusions regarding a causative association with hand eczema are limited.

With 8·5 years of follow-up the possibility of recall bias cannot be excluded. Respondents with a short eruption of hand eczema at the beginning of the follow-up period might forget about it. Also, the natural history of hand eczema is characterized by disease-free intervals and more or less frequent recurrent eruptions. Thus it is important not to register a subsequent eruption as an incident case. Because of the relatively short follow-up period, we do not believe that this is an important problem in this study.

In conclusion, hand eczema is still a frequent skin disease. Genetic factors are confirmed important risk factors, whereas sex or age did not influence the risk. Also, positive patch test, atopic dermatitis and wet work were associated with an increased risk of hand eczema. The lifestyle factors, smoking and alcohol, were not significant risk factors. The results on positive patch test, atopic dermatitis, wet work, smoking and alcohol should be interpreted with caution as the data were collected after the development of hand eczema and may be biased.

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Clinical characteristics and consequences of hand eczema

– an eight year follow-up study of a population-based twin cohort

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Summary

Background Few population-based clinical follow-up studies on hand eczema are reported.

Objectives The aim of this study was to characterize clinical symptoms and to examine occupational and medical consequences as well as persistence of hand eczema in a population-based twin cohort.

Patients/Methods A total of 274 individuals with and without hand eczema were examined, patch tested and interviewed in 1997-98 and 2005-06. Data on 188 individuals with hand eczema in 2005-06 was analysed.

Results Erythema and scaling were the most frequent symptoms and fingers and palms were most often affected. Mean HECSI (Hand Eczema Severity Index)-score in individuals with clinical symptoms was 12.0. Sick leave was reported by 12.4%; job change by 8.5%. Being in the lowest socio-economic group and atopic dermatitis were risk factors for sick leave (OR=5.6; 95% confidence interval [95% C.I.] 1.5-22.9 and OR=2.9; 95% C.I. 1.0-8.1). The majority (63.4%) had seen a doctor at least once, and atopic dermatitis was a risk factor for more than one visit (OR=3.0; 95% C.I. 1.4-6.4). Duration of > 10 years was a risk factor for persistence of symptoms, which was reported by 67.7%.

Conclusions The clinical picture and consequences of hand eczema varies, however, the majority experience chronic symptoms.

Key words: hand eczema, follow-up, clinical study, consequences

Introduction

Epidemiological studies on hand eczema have repeatedly confirmed that hand eczema is a frequent dermatological condition with a 1-year prevalence around 10% and a lifetime prevalence around 20% (1, 2). Symptoms may vary from mild to severe, affecting quality of life and potentially resulting in sick leave and/or job change (3, 4). Disease duration is variable; however a tendency to chronicity is characteristic and symptoms may persist for many years or recur after disease free intervals (5).

Follow-up studies on hand eczema are regularly reported; however, only few are population-based (5-7). The majority of studies describe selected groups, such as patients from dermatological departments and/or with occupational dermatitis (8-11). The disease pattern and prognosis in these selected groups may vary from what is seen in the general population. Moreover, many follow-up studies are solely questionnaire-based and do not include clinical assessment of severity or symptoms.

The aim of this follow-up study was to characterize clinical symptoms and examine occupational and medical consequences as well as persistence of hand eczema in a population-based twin cohort. The cohort consisted of twins; however, a twin design was not utilized in the study.

Materials and methods

Study population

In 1997-98 a total of 1076 twin individuals born between 1953 and 1976 participated in a clinical examination (denoted "first examination"), interview and patch testing (12,13). The twin individuals were ascertained from a population-based twin cohort of 5610 same-sex twins participating in a questionnaire survey in 1996 on hand eczema (2). Results from a follow-up questionnaire study in 2005 have recently been published (14). To be invited to the first examination both twins in a twin pair should have returned the questionnaire and at least one of them should have reported symptoms of hand eczema or hand eczema. In addition, both twin individuals in a twin pair had to live within 60 km from Copenhagen.

In total, 659 twin individuals had self-reported hand eczema themselves or had a co-twin with self-reported hand eczema in 1997-98. The remaining twin individuals reported one or more symptoms of hand eczema. Invitation to participate in a new clinical examination (denoted "second examination"), structured interview and patch testing between May 2005 and June 2006 was restricted to twin individuals with self-reported hand eczema themselves or with a co-twin with self-reported hand eczema in 1997-98 . Addresses were obtained from the Danish Civil Registration System on 605 twin individuals. The remaining twin individuals had a protected address, had emigrated or died. Participants were enrolled after informed consent, in compliance with the principles of the Helsinki Declaration.

Clinical examination and interview

The hand eczema severity index (HECSI) was used to score symptoms of hand eczema at the second examination (15). The score is based upon registration of extension (i.e. fingertips, fingers, palm of hands, back of hands and wrists) and symptoms (i.e. erythema, infiltration/papules, vesicles, fissures, scaling and oedema) graded as no skin changes, mild disease, moderate disease and severe disease. The minimum score is 0 and the maximum score is 360.

In the structured interview participants answered questions on self-reported hand eczema, atopic dermatitis, socio-economic status, age at onset, time of last hand eczema eruption, current exposure to wet work, current glove use and current frequency of hand washing. Also, questions on ever having been on sick leave, ever having changed job due to hand eczema or ever to have notified the Danish National Board of Industrial Injuries and number of medical consultations ever were asked.

Patch testing

All participants were patch tested with the ready-to-use TRUE Test® system (Mekos Laboratories ApS) panel 1 and 2 in 1997-98 and 2005-06. Reading of the patches was done on day three according to the International Contact Dermatitis Research Group's guidelines (16).

Definitions

Hand eczema. A diagnosis of hand eczema was based on a positive answer to a question on self-reported hand eczema (Have you ever had hand eczema?), given either in the questionnaire survey in 1996 or at the second examination. *Age at onset.* Participants were asked about age at onset of hand eczema at the first examination. Participants with onset in the follow-up period were asked at

the second examination. Participants were subdivided into two groups with age at onset ≤ 15 years and > 15 years. This division was arbitrarily chosen as a way to distinguish between individuals with onset in childhood and onset in adulthood. *Socio-economic status* was based on Socio (Statistics Denmark's Socio-economic classification), 1st edition 1997. This classification system is based upon educational skills. Participants were divided into three groups: (1) highest/medium level (minimum 15 years of educational training), (2) basic level (11-14 years of educational training) and (3) lowest level (up to 10 years of educational training and/or unemployed or pensioner). *Atopic dermatitis* was defined according to the U.K. Working Party's Diagnostic Criteria (17). *Persistent hand eczema* was defined as hand eczema within the last year prior to the second examination. Participants having their last hand eczema eruption more than one year prior to the first examination were excluded from the analysis on persistence of hand eczema. *Wet work* was defined as skin exposed to liquids more than 2 hours per day, or use of occlusive gloves more than 2 hours per day, or very frequent hand washing (>20 times/day) (18). *Duration* at the first examination was calculated by subtracting year at onset from year at first examination, thus ignoring periods with complete healing in between. Participants were subdivided into two groups having had hand eczema ≤ 10 years and > 10 years, respectively.

Statistical analyses

Data management, descriptive statistical analyses, and the chi squared (χ^2) test used in the drop-out analysis, were done in SPSS version 13.0. Logistic regression analyses were performed with Stata Statistical Software. All P-values are 2-sided and a 5% significance level was used. As the twins in a twin pair are not statistically independent, the confidence intervals were corrected for intra-twin correlation using the option "cluster" in Stata.

In a multiple logistic regression analysis the potential influence of sex, zygosity, age at onset, socio-economic status, atopic dermatitis and positive patch test in 1997-98 on the risk of sick leave and medical consultations was explored. Likewise the influence of sex, zygosity, age at onset, socio-economic status, atopic dermatitis, positive patch test in 1997-98, current wet work and duration of hand eczema at the first examination on the risk of persistent hand eczema was evaluated in a multiple logistic regression analysis.

Results

A total of 274 twin individuals volunteered to participate in the clinical examination and interview, resulting in a participation rate on 41.5% (274/659).

Hand eczema and descriptive data

174 individuals had self-reported hand eczema at the first examination in 1997-98. Fourteen new cases was detected at the second examination, thus the total number of individuals with self-reported hand eczema in 2005-06 was 188. The following data relates to these 188 individuals. The group comprised 128 women and 60 men, with a mean age of 42 years (SD 6.4). The group comprised 34 twin pairs and 120 single twin individuals (101 monozygotic twin individuals, 75 dizygotic twin individuals and 12 with unknown zygosity). The mean follow-up period was 8.6 years (range 7.4-9.4). Regarding socio-economic status, 72/188 (38%) was in the highest/medium level, 87/188 (46%) was in the basic level and 29 (15%) in the lowest level. A total of 58/188 (30.9%) had wet work according to the definition.

Drop-out analysis

Drop-out analysis of twin individuals participating in the second examination (274) versus those where one or both twin individuals had self-reported hand eczema in 1997-98 (659) revealed no statistically significant differences regarding sex, zygosity, hand eczema status, co-twins hand eczema status, patch test status or atopic dermatitis status (data not shown). Age was the only statistically significant factor influencing willingness to participate. The group was subdivided into three groups of equal size. Only 35% from the youngest age group (born 1969-76) participated, in the middle group (born 1961-68) 41% participated, whereas 50% from the oldest age group (born 1953-1960) volunteered to the study (p=0.009).

Clinical symptoms and severity

In total, 77/188 (41.0%) had one or more clinical symptoms of hand eczema at the second examination. Erythema and scaling were the most frequently encountered clinical symptoms and fingers (excluding fingertips) and palms were most often affected (see fig. 1 and 2). A total of 98/188 (56.9%) had symptoms of hand eczema at either one of the examinations. The mean HECSI score was 4.9 (SD 13.3, range 0-120). If only individuals with clinical symptoms were counted, the mean HECSI score was 12.0 (SD 18.7). The mean HECSI score in males and females was 4.0 (SD 7.2)

and 5.4 (SD 15.4), respectively. The mean HECSI score in individuals with atopic dermatitis was 9.9 (SD 25.8), as opposed to a mean HECSI score of 3.6 (SD 6.6) in individuals without atopic dermatitis.

Atopic dermatitis

A subgroup of 39/188 (20.7%) had current or previous atopic dermatitis.

Patch test results

The frequency of a positive patch test in 1997-98 and 2005-06 was 53/188 (28.2%) and 56/185 (30.3%), respectively. Three participants were not patch tested in 2005-06 due to breastfeeding and immunosuppressive therapy. A total of 40 (21.6%) had a positive patch test to nickel sulphate. Further details on the results of the patch testing are presented elsewhere (19).

Occupational consequences

A subgroup of 23/185 individuals (12.4%) had ever been on sick leave because of hand eczema. When summing all sick leave periods, 4 individuals (2.2%) reported sick leave for less than a week, 8 (4.3%) for 1-2 weeks, 5 (2.7%) for 3-5 weeks and another 6 (3.2%) for more than 6 weeks. Three did not answer the question. Being in the group with the lowest socio-economic status, compared to the group with the highest socio-economic status and atopic dermatitis, were the only statistically significant factors influencing the risk of ever having been on sick leave (OR=5.6; 95% C.I. 1.4-22.3 and OR=2.9; 95% C.I. 1.0-8.1), see table 1. A smaller group of 16 individuals (8.5%) had ever changed their job because of hand eczema. All, but one, had changed job once. Notification to the Danish National Board of Industrial Injuries Registry was reported by 19 individuals (10.1%).

Medical consultations

The majority had ever seen a doctor because of hand eczema (118/186; 63.4%). Two individuals did not remember. An approximately equal number (25.3% and 22.6%) had seen a doctor only once or 2-5 times respectively, whereas a smaller group (15.6%) had seen a doctor more than 5 times. Atopic dermatitis was associated with an increased risk of reporting more than one medical consultation compared to no or just one medical consultation (OR=3.0, 95% C.I. 1.4-6.4), see table 1.

Persistence of hand eczema

Of those with self-reported hand eczema within one year prior to the first examination (N=142), a total of 96 (67.6 %) still had hand eczema within the last year prior to the second examination. Individuals with duration of hand eczema > 10 years at the first examination had an increased risk of persistent hand eczema (OR 2.5; 95% C.I.1.0-6.0). Also, in the analysis being a dizygotic twin was associated with an increased risk of persistence, when compared to monozygotic twins (OR=2.6; 95% C.I. 1.2-5.4), see table 2.

Discussion

The present follow-up study confirmed that hand eczema is a chronic disease, with 67.6% still reporting persistence of hand eczema within the last year after 8 years of follow-up.

Erythema and scaling were the most frequently reported symptoms and palms and fingers were the most frequently affected areas. This is in line with a previous report (20). Severity scoring, judged by symptoms and extension, was generally mild. Recently, a number of different hand eczema severity scores based on clinical evaluation of symptoms and extension or based on photographs have been developed, but none has yet been employed in clinical epidemiological studies or gained general acceptance (15, 21, 22). The HECSI score was used in this study and proved practicable; however, a corresponding clinical grading (fx mild, moderate and severe hand eczema) has not yet been defined.

Atopic dermatitis was defined according to the U. K. Working Party's Diagnostic Criteria and 20.7% met the criteria. Meding and Swanbeck in a population-based cohort study on hand eczema, found a similar estimate, namely that 22% had atopic hand eczema (23). Also, the frequency of contact sensitization of 30.3% found in this study is comparable to a previous population-based study, reporting a positive patch test in 32% of individuals with hand eczema (24).

Possible consequences of hand eczema are sick leave and/or change of job. In the present study twelve percent had ever been on sick leave due to hand eczema. The proportion having ever been on sick leave varies among different studies, reflecting among other things a variable length of follow-up and difference in study populations. Also, variation in social insurance systems between countries and the state of the market influences the sick leave rate, which may thus also vary over time in

the same country. Occupational hand eczema often causes sick leave (4). Meding et al in a 12-year follow-up study on occupational hand eczema found that 48% had been on sick leave for at least a week, whereas in a population-based 15-year follow-up study only 6% had been on sick leave in the follow-up period (5, 9). In this study 8.5% stated that they had changed their job due to hand eczema. The corresponding numbers in the two above-mentioned studies from Meding et al were 44% and 3%. One in ten reported that the hand eczema had been notified to the Danish National Board of Industrial Injuries Registry. To our knowledge no data on the proportion of notified cases in a population-based cohort has previously been published. The proportion of notified cases will likely vary greatly between countries, due to differences in insurance systems and registries.

Although, the majority of individuals with hand eczema had seen a physician, one-third did not seek medical advice and 25.3% had only one contact. This finding is in agreement with data previously reported (25). Thoughts on how these individuals manage their disease, the reasons for not seeing a doctor and what characterize their hand eczema can only be speculative. Atopic dermatitis was associated with an increased risk of more than one medical consultation, also in agreement with previous findings (25).

In total, 67.6% reported persistent hand eczema, defined as hand eczema within the last year of the second examination. A 12-year follow-up study on farmers found that 40% still had symptoms within the last year; the 12-year follow-up study on occupational skin disease found that 70% reported symptoms within the last year and the 15-year population-based study estimated that 44% experienced symptoms within the last year (5, 9, 11). The seemingly high frequency of persistent hand eczema in this study may partly be explained by the shorter follow-up period. Duration above 10 years at start of follow-up was a risk factor for persistent hand eczema. In a study on occupational chromate dermatitis, duration of symptoms for more than 12 months before diagnosis of chromate sensitivity was associated with persistence of dermatitis (10). The significantly increased odds ratio for dizygotic twins concerning persistent hand eczema was unexpected. The limited sample size is a probable cause.

The diagnosis of hand eczema relied on a questionnaire-based self-report of hand eczema using a question very similar to one, which has previously been validated and shown to have a high specificity, but less sensitivity (26). Misclassification, especially mild cases misclassified as not having hand eczema and thus not being invited to participate, is possible. The opposite scenario, i.e. cases without hand eczema misclassified as having hand eczema is less likely; however, it may occur if

individuals with other skin diseases on the hands (i.e. tinea, psoriasis, pustulosis-palmoplantaris) are classified as having hand eczema (26). In approximately half of the individuals the diagnosis could be confirmed by objective symptoms at either one of the clinical examinations, and in the remaining individuals verification of the diagnosis was attempted by asking thoroughly to history, symptoms and treatment. However, the self-reporting of a disease may be influenced by an increased awareness of the disease, which can be anticipated in a twin pair with an affected member. In that case sensitivity and specificity may change. A decrease in specificity is of greatest concern and cannot be excluded. A clinical diagnosis would have been preferable in all cases, but was impossible, as many of the cases in the sample only had historic symptoms.

Selection bias is a concern in this study due to the limited participation rate. Drop-out analysis revealed no differences between participants and non-participants with regard to sex, zygosity, hand eczema status, co-twins hand eczema status, atopic dermatitis status or patch test status. However, a selection bias with a tendency for more severe cases and cases with recent symptoms to attend cannot be excluded. Thus, the proportion with persistent symptoms, occupational and medical effects may be increased in this sample and the analysis on factors associated with persistence may be hampered by loss to follow-up of already recovered cases. Supporting this, Meding et al, in a drop-out analysis of individuals participating in a dermatological examination, found that more individuals had continuous symptoms among those attending in the study than among those not attending (1). Also, recall bias may influence the results, as information on occupational and medical effects, age at onset, year of last eruption and atopic dermatitis was based on questions answered by the participants. The effect of a potential recall bias is more unpredictable and both under- and overestimation of the estimates is possible.

Specific statistical twin analyses were not performed in this study. Extrapolation of the results from a twin study to the general population requires that the twin population is representative of the background population. The study population was drawn from twin cohorts in the Danish Twin Registry, which is based on the Danish Civil Registration System and covers 74.4% of all twins born 1953-67 (incl.) and 97.4% of those born 1968-1982 (incl.) (27, 28). The prevalence of hand eczema and atopic dermatitis in twins has been shown to be comparable to prevalences in non-twin populations (2, 14). Whether twin individuals have an increased frequency of sick leave or medical consultations due to hand eczema or persistent hand eczema compared to a non-twin population is unknown. However, the results obtained in this study are comparable to previous reports.

In conclusion, this population-based study confirms the diversity in clinical characteristics of and consequences of hand eczema. A total of 12.4% had ever been on sick leave, while 8.5% had changed their job. The majority had seen a doctor at least once (62.7%). Low socio-economic status and atopic dermatitis were risk factors for sick leave and atopic dermatitis in addition a risk factor for more than one doctor visit. Persistent hand eczema was reported by 67.7%, and long duration (>10 years) was a risk factor for persistent hand eczema.

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Conflict of Interest The authors declare no conflict of interest.

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Figure 1. Clinical symptoms of hand eczema observed at clinical examination (N=77).

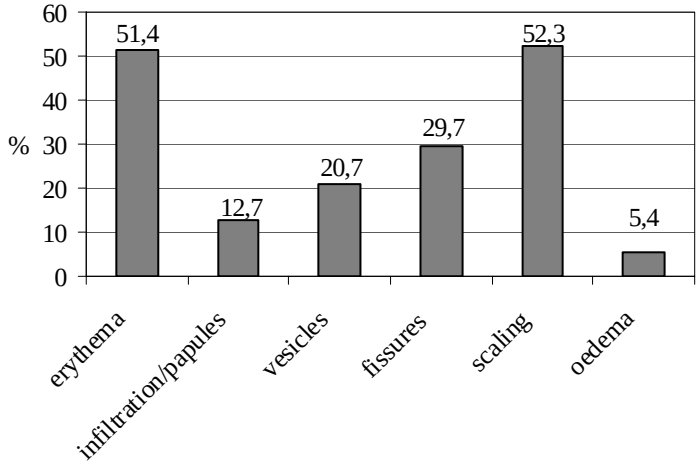

Figure 2. Extension of clinical symptoms observed at the clinical examination (N=77)

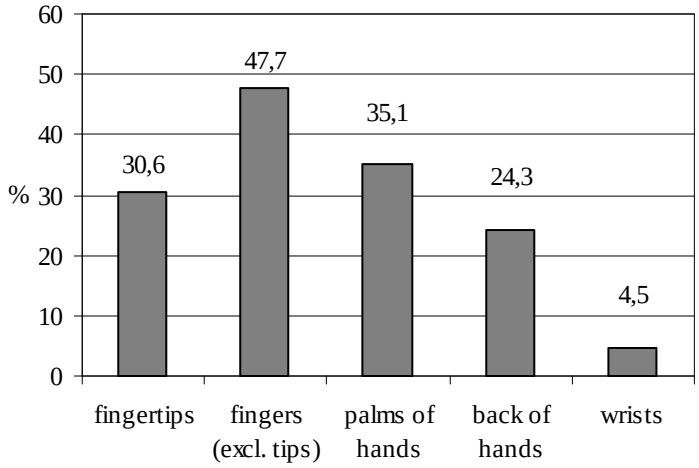

Table 1. Results of the multiple regression analysis on sick leave and more than one medical consultation due to hand eczema

		Sick leave ever[1] (N=185)			More than one medical consultation[2]		
		N (total)	**OR[3] (95% C.I.)[4]**	**P-value**	**N (total)**	**OR[3] (95% C.I.)[4]**	**P-value**
Sex	**Male (ref)**	4 (59)	1	0.278	21 (60)	1	0.552
	Female	19 (126)	2.1 (0.6-7.7)		50 (126)	1.2 (0.6-2.6)	
Zygosity	**Monozygotic (ref)**	9 (74)	1		33 (75)	1	
	Dizygotic	13 (99)	0.8 (0.3-2.1)	0.837[5]	33 (99)	1.5 (0.8-2.9)	0.327[5]
	Unknown zygosity	1 (12)	1.5 (0.2-11.9)		5 /12	2.1 (0.6-7.3)	
Age at onset	**≤15 years (ref)**	5 (49)	1	0.543	21 (49)	1	0.398
	>15 years	18 (136)	1.4 (0.5-4.4)		50 (137)	0.7 (0.4-1.5)	
Socio-economic status	**Highest (ref)**	4 (72)	1		23 (72)	1	
	Basic	12 (86)	3.0 (0.8-10.6)	**0.050[5]**	33 (86)	1.4 (0.7-2.8)	0.163[5]
	Lowest	7 (27)	5.6 (1.4-22.3)		15 (28)	2.5 (1.0-6.4)	
Atopic dermati-	**No (ref)**	14 (147)	1	**0.049**	48 (147)	1	**0.006**
	Yes	9 (38)	2.9 (1.0-8.1)		23 (39)	3.0 (1.4-6.4)	
Positive patch	**No (ref)**	16 (133)	1	0.516	52 (133)	1	0.408
	Yes	7 (52)	0.7 (0.2-2.1)		19 (53)	0.7 (0.3-1.5)	

[1]Sick leave ever due to hand eczema.
[2]More than one medical consultation ever due to hand eczema.
[3]Odds ratio.
[4]95% confidence interval.
[5]Test if equal odds in all three groups.
[6]In 1997-98.

Table 2. Results of the multiple regression analysis on persistent hand eczema.

		Persistent hand eczema[1] (N=142)		
		N (total)	**OR[2] (95% C.I.)[3]**	**P-value**
Sex	**Male (ref)**	32 (41)	1	0.098
	Female	64 (101)	0.4 (0.2-1.2)	
Zygosity	**Monozygotic (ref)**	34 (60)	1	
	Dizygotic	57 (76)	2.6 (1.2-5.4)	**0.041[4]**
	Unknown zygosity	6 (6)	3.4 (0.3-42.4)	
Age at onset	**≤ 15 years (ref)**	23 (34)	1	0.316
	> 15 years	73 (108)	1.7 (0.6-4.7)	
Socioeconomic status	**Highest (ref)**	35 (52)	1	
	Basic	44 (69)	1.1 (0.5-2.6)	0.226[4]
	Lowest	17 (21)	2.7 (0.8-8.8)	
Atopic dermatitis	**No (ref)**	72 (106)	1	0.981
	Yes	24 (36)	1.0 (0.4-2.5)	
Positive patch test[5]	**No (ref)**	72 (102)	1	0.284
	Yes	24 (40)	0.6 (0.2-1.5)	
Wet work	**No (ref)**	65 (94)	1	0.830
	Yes	31 (48)	1.1 (0.5-2.6)	
Duration	**≤ 10 years (ref)**	39 (65)	1	**0.044**
	> 10 years	57 (77)	2. (1.0-6.0)	

[1]Hand eczema within the last year prior to the second examination. Analysis restricted to individuals with hand eczema within one year prior to the first examination.
[2]Odds ratio.
[3]95% confidence interval.
[4]Test if equal odds in all three groups.
[5]In 1997-98.

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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 retesting 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%) 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, $n = 274$, total	Present study, $n = 270$					Comparison: previous and present studies		
		Women, $n = 180$	Men, $n = 90$	Total	Hand eczema, $n = 185$	No hand eczema, $n = 85$	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)
p-t-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, ethylendiamine 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.
[a]Percentages 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 population 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 indicates 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 (Cl+ 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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Filaggrin null alleles are not associated with hand eczema or contact allergy

Running head: Filaggrin null alleles, hand eczema and contact allergy

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Summary

Background The filaggrin protein is a key component of stratum corneum and homo- or heterozygotes for the filaggrin variant alleles R501X and 2282del4 have varying degrees of impaired skin barrier. The variant alleles have repeatedly been shown to be associated with atopic dermatitis. Any possible association with hand eczema or contact allergy are unexplored.

Objective In this study, associations between the variant alleles, hand eczema, contact allergy and atopic dermatitis were explored.

Patients/Methods In total, 183 adult individuals participated in a clinical examination of the hands, patch testing and filaggrin genotyping. Children without any evidence of atopic dermatitis from the COPSAC study were used as controls. The chi squared (χ^2) test was used for comparison of allele frequencies.

Results The majority (73%) had hand eczema, 25% had contact allergy and 14% had a diagnosis of atopic dermatitis. The association between atopic dermatitis and the filaggrin variant alleles was confirmed (OR=3.5, p=0.015). Allele frequencies in individuals with hand eczema or contact allergy were not statistically significantly increased.

Conclusion There was no association between the variant alleles and hand eczema or contact allergy.

Key words: atopic dermatitis, contact allergy, filaggrin, hand eczema

Background

Filament aggregating protein (filaggrin) is an essential component in the terminal differentiation of the epidermis and formation of the stratum corneum. Profilaggrin, the precursor of filaggrin, accumulate in the keratohyalin granules formed in the granular layers of the epidermis. During cornification keratin filaments in keratinocytes are aggregated by filaggrin, resulting in a flattening of the keratinocytes and eventually formation of the cornified cell envelope, which is crucial for the skin barrier function[1]. The skin barrier provides protection against water loss and penetration of chemical, infectious and allergenic agents.

Homozygotes or compound heterozygotes for the two loss-of-function mutations (null alleles) R501X and 2282del4 in the gene encoding filaggrin have a complete loss of filaggrin products and present clinically with ichtyosis vulgaris, characterized by dry scaly skin[2]. The mode of inheritance is semidominant with variable penetrance; heterozygotes may present with a mild form of ichtyosis vulgaris or without symptoms.

A number of studies have recently established a strong association between the filaggrin variant alleles and atopic dermatitis[3], in particular atopic dermatitis associated with asthma and allergic rhinitis[3;4], IgE-sensitization[5], early onset[6] and persistence into adulthood[7].

Hand eczema is a frequent, often chronic relapsing disease, with a heterogeneous etiology, including irritant and allergic contact dermatitis, atopic dermatitis, mixed forms and minor groups with vesicular and hyperkeratotic hand eczema. Atopic dermatitis is one of the main risk factors for hand eczema[8]. Genetic risk factors significantly influences the risk of developing hand eczema[9], even in the absence of atopic dermatitis[10;11]. Genetic markers for hand eczema have not yet been identified and any possible association to the filaggrin variant alleles is unexplored. An impaired skin barrier facilitates the penetrance of allergens, but whether the presence of mutations in the gene encoding filaggrin is associated with an increased frequency of contact allergy is unknown.

The primary aim of this study was to investigate whether any relationship exists between the filaggrin variants R501X and 2282del4 and hand eczema. Secondly, any associations between the variant alleles and contact allergy and atopic dermatitis were explored.

Materials and Methods

Study population

In 1996 a cohort of 6666 same-sex twin individuals born between 1953 and 1976 and living on Zealand or its neighbouring islands was drawn from the Danish Twin Registry and received a short questionnaire on hand eczema[9]. A total of 5610 twin individuals responded. Twin pairs where one or both twin individuals had self-reported hand eczema or reported symptoms of hand eczema in the questionnaire and lived within 60 km from Copenhagen were invited to a clinical examination and patch test in 1997-98[12]. A total of 1076 twin individuals participated. In 2005 all twin pairs where one or both had self-reported hand eczema in 1997-98 were identified (659 individuals). Addresses were available on 605 twin individuals and they were invited by mail to a second clinical examination and patch test in 2005-06 (Figure 1). All individuals gave their written, informed consent for participation in compliance with the principles of the Helsinki Declaration.

Hand eczema and atopic dermatitis

A diagnosis of hand eczema was based on a positive answer to a question on self-reported hand eczema (Have you ever had hand eczema?)[13], given either in 1997-98 or at the present examination. Fifteen individuals with self-reported hand eczema in 1997-98 denied hand eczema at the present examination. Some of them had other diagnoses such as psoriasis and polymorphic light eruption on the hands. In case of present hand eczema, symptoms (scaling, erythema, vesicles, papules, fissures, and edema) were recorded. The U.K. Working Party's Diagnostic Criteria were used to define whether participants ever had had atopic dermatitis[14].

Filaggrin genotyping

Venous blood samples or mouth swaps were collected from the twin individuals and kept at -80ºC. DNA was prepared from blood samples and mouth swabs using QIAamp -96 DNA procedures (Qiagen GmbH, Hilden, Germany). Genotyping for R501X and 2282del4 was performed by TAQMAN allelic discrimination assays as previously described[3;15].

Patch testing

The ready-to-use TRUE Test® system panel 1 and 2 (Mekos Laboratories AS) was send by mail to all participants. Patches were placed on the back by the participants three days in advance of the scheduled examination and removed after 2 days. Reading of the patches was done on day three according to the International Contact Dermatitis Research Group guidelines[16].

Control groups

At first, allele frequencies in twin individuals with hand eczema were compared with twin individuals without hand eczema, and likewise twin individuals with and without contact allergy and atopic dermatitis were compared. As the number of twin individuals without hand eczema and contact allergy was limited, allele frequencies in the twin subgroups with hand eczema and contact allergy were also compared to a group of 189 children without atopic dermatitis (91 male and 98 female) all born to Danish mothers with asthma. The children are currently being followed from birth in a prospective longitudinal follow-up study (the COPSAC study[3;17]). Atopic dermatitis in the COPSAC study was defined using the criteria of Hanifin and Rajka[18]. Finally, in an analysis restricted to the subgroup of twins with hand eczema allele frequencies in the subgroup with and without atopic dermatitis were compared.

Statistical analysis

When data on both twin individuals in a twin pair was available, one twin individual was randomly excluded from the analysis, thus leaving 183 twin individuals for analysis (Figure 1). Allele frequencies were compared in subgroups of twins and in the COPSAC subgroup using the chi squared (χ^2) test. Both variants were in Hardy-Weinberg equilibrium in the twin cohort, the twin subgroups and in the COPSAC subgroup. The chi squared (χ^2) test was used in the drop-out analysis. SPSS version 13.0 was used for statistical analyses.

Results

A total of 274 twin individuals participated. DNA genotyping was successful in 263 individuals and 183 twin individuals (70 monozygotic, 103 dizygotic and 10 with unknown zygosity) were selected for analysis (see statistical analysis). The following descriptive data apply to the 183 individuals.

The mean age was 41 years (SD 6.6) and the male/female proportion was 64/119. The majority (72.7%) had had hand eczema and in this group, clinical signs of hand eczema were found in 41%. At least one positive patch test was detected in 24.6%; the frequency in women and men was 31.9% and 10.9%, respectively. Nickel was the primary allergen responsible for sensitization (data not shown). A total of 14.2% had current or previous history of atopic dermatitis. All twin individuals with atopic dermatitis reported at least one episode of hand eczema. The frequency of atopic dermatitis in the hand eczema group was 19.5%.

The overall allele frequencies of R501X and 2282del4 in the twin cohort were 3.3% for both variants (yielding carrier frequencies of 6.6%). As there were no compound heterozygotes the combined carrier frequency was 13.1%. There were no homozygotes in the twin cohort. Highest allele frequencies were found in the twin subgroups with atopic dermatitis and contact allergy, 23.1% and 15.6%, respectively. Allele frequencies in the twin cohort, the twin subgroups and the COPSAC group are shown in table 1.

No association between the phenotype with hand eczema and the two variant alleles was found. Also, no association between contact allergy (positive patch test) and the variant alleles could be demonstrated. The increased combined carrier frequency in individuals with atopic dermatitis did not reach statistical significance when compared to twin individuals without atopic dermatitis. Statistical results from the comparisons are displayed in table 2.

Allele frequencies in the twin subgroups with hand eczema or contact allergy were not statistically significantly different from allele frequencies in the COPSAC subgroup of children without atopic dermatitis. Comparison of the twin subgroup with atopic dermatitis with the COPSAC subgroup reached statistical significance. See table 2 for details.

In the subanalysis, restricted to twin individuals with hand eczema, comparison of the combined allele frequency in individuals with atopic dermatitis (23.1%) with the subgroup without atopic dermatitis (10.1%) was borderline statistically significant (OR= 2.6 [95% confidence interval 0.87—7.91]; χ^2=3.072; p=0.080).

Drop-out analysis of the 274 twin individuals participating in the present study versus those where one or both twin individuals had self-reported hand eczema in 1997-98 (659 twin individuals) revealed no statistically significant difference regarding sex, zygosity, hand eczema status in 1997-98, co-twins hand eczema status in 1997-98, patch test status or atopic dermatitis status (data not

shown). Age was the only statistically significant factor influencing willingness to participate. When subdividing the twin individuals into three groups, 35% from the youngest age group participated, in the middle group 41% participated, whereas 50% from the oldest age group volunteered to the study (p=0.009).

Discussion

The influence of genetic factors independent of atopic dermatitis on the risk of hand eczema was recently confirmed[10]. Many candidate genes for atopic dermatitis has been proposed and investigated, but the filaggrin null alleles, R501X and 2282del4, are the first to be successfully replicated in a number of studies[19]. The epidermal defect caused by the variants could possibly be of etiological importance in other skin diseases, characterized by a compromised skin barrier or where a compromised skin barrier can be a trigger factor.

We investigated a possible association between the variant alleles and the phenotype with hand eczema. In this population-based, but selected group of individuals, with a high prevalence of hand eczema and atopic dermatitis, we found an overall combined carrier frequency of 13.1%. This is higher than other reported frequencies in the background population between 8.8 % and 9.6 %[3;6;7]. Two studies found considerably lower combined carrier frequencies in the control groups. Weidinger et al reported 6.1 % and Marenholz et al found only 5.1 % carrying the variant alleles, however, in the last study, a "hyper-normal" control group was selected, as none of them had any allergy[4;20].

The twin subgroup with hand eczema did not exhibit a higher mutation frequency than the group without hand eczema; however the (control) group without hand eczema was small, limiting conclusions. Comparison with a subgroup of children without atopic dermatitis from a high-risk cohort of children born of mothers with asthma (COPSAC) also failed to find any statistically significant difference.

A defect skin barrier facilitates induction and elicitation of contact allergy. Kligman et al found increased rates of sensitization after pre-treatment with an irritant or after combined exposure[21]. Also, pre-treatment of the skin with an irritant or combined exposure to an irritant and an allergen lowers the threshold for elicitation of contact allergy or increases the patch test response[22;23]. The impaired

skin barrier caused by the variant alleles could possibly increase the risk of contact allergy. We did not find any statistically significant association between the variant alleles and contact allergy in the analysis of the total twin cohort or when compared with the COPSAC group.

The allele frequencies in twin individuals with hand eczema or contact allergy were increased compared to the COPSAC cohort, 12.8% and 15.6% versus 7.4%, respectively. The negative outcome of the association analyses may be due to insufficient power and using a larger population-based control group may yield a different result. Thus a possible association between the variant alleles and hand eczema or contact allergy cannot be entirely excluded. Three of the seven individuals with a variant allele and contact allergy also had atopic dermatitis.

Contact allergy is diagnosed by means of patch testing. Some limitations apply to this study. Errors in the patch test procedure cannot be entirely excluded, as participants applied and removed the patches themselves. Secondly, as reading was done only on day three, late reactions can be missed.

It has been demonstrated that between 3 and 8.2% of reactions become positive on day 6 or 7[24;25]. Furthermore, individuals were only tested with 20 allergens, including the most frequent sensitizers. A negative test is obviously not a proof of absence of contact allergy. However, these standard allergens have been shown to detect 77 to 95% of all contact allergies in departments specialized in contact dermatitis[26].

In the twin subgroup with atopic dermatitis the combined carrier frequency was 23.1%, however, this was not statistically significantly different from the frequency in individuals without atopic dermatitis, even though the frequency in this group was much smaller (11.4%). A likely explanation is a lack of power, since the group with atopic dermatitis comprised only 26 individuals. We confirmed the association between atopic dermatitis and the filaggrin variant alleles when comparing with the COPSAC subgroup (OR=3.5, p=0.015). In the subanalysis including only individuals with hand eczema, the comparison of subgroups with and without atopic dermatitis was borderline statistically significant. Thus the filaggrin null alleles could be a potential genetic marker for increased risk of atopic hand eczema (in individuals with atopic dermatitis). It is previously shown that the risk of hand eczema increases with the severity of atopic dermatitis[8].This will need further investigation in a study including individuals with atopic dermatitis but without hand eczema.

Opposed to many of the previous hospital-based studies on association between atopic dermatitis and the filaggrin variant alleles, this study was population-based. Thus, our group with atopic der-

matitis may represent a greater spectrum of disease severity including phenotypes with milder symptoms, compared to earlier studies. Weidinger et al actually demonstrated that the 2282del4 mutation and the combined genotype was statistically significantly associated with a more severe phenotype (SCORAD>31)[20].

The U.K. Working Party's Diagnostic Criteria have been thoroughly validated, though mostly, but not entirely in children. In this study the possibility of recall bias is considerable. Possibly, mild cases with symptoms restricted to early childhood have been forgotten and thus missed. Such a misclassification could change the results in both directions depending on whether those in question were carriers of the variant alleles or not and whether they had hand eczema or not.

A well-defined phenotype is essential in genetic association studies. In this study the diagnosis of hand eczema was based on a question on self-reported hand eczema. A similar question is validated and has shown high specificity, but less sensitivity[27]. In the present context a high specificity is preferable. Furthermore the diagnosis could be confirmed in 41% of cases due to visible signs of hand eczema at the examination.

The COPSAC subgroup of children without atopic dermatitis was chosen as a secondary control group due to the limited number of twin individuals without hand eczema and due to availability. A more suitable control group would be an adult group without hand eczema and contact allergy, matched on sex and atopy status.

Individuals with hand eczema comprise a very heterogeneous group both regarding aetiology, severity and prognosis. Finding a single candidate gene influencing all subtypes may turn out to be a difficult task, even though the inflammatory processes may be similar. Two cytokine gene polymorphisms have been identified as being of importance for the development of allergic contact dermatitis; IL16-295 and TNFA-308 respectively[28;29]. The latter was also present in increased frequency in individuals with a low irritation threshold and may thus also be a marker of increased risk of irritant contact dermatitis[30].

In conclusion, no association between the filaggrin null alleles and hand eczema or contact allergy overall could be demonstrated, however, insufficient power is a consideration. The association between atopic dermatitis and the filaggrin variant alleles was confirmed in the comparison of the individuals with atopic dermatitis with the COPSAC subgroup without atopic dermatitis. In the subanalysis, including only individuals with hand eczema, an almost statistically significant increased

frequency in individuals with atopic dermatitis was seen. This suggests that the filaggrin null alleles in future could be used as a potential marker for increased risk of hand eczema in patients with atopic dermatitis. However, this hypothesis needs further investigation.

Conflicts of interests

The authors declare no conflicts of interest.

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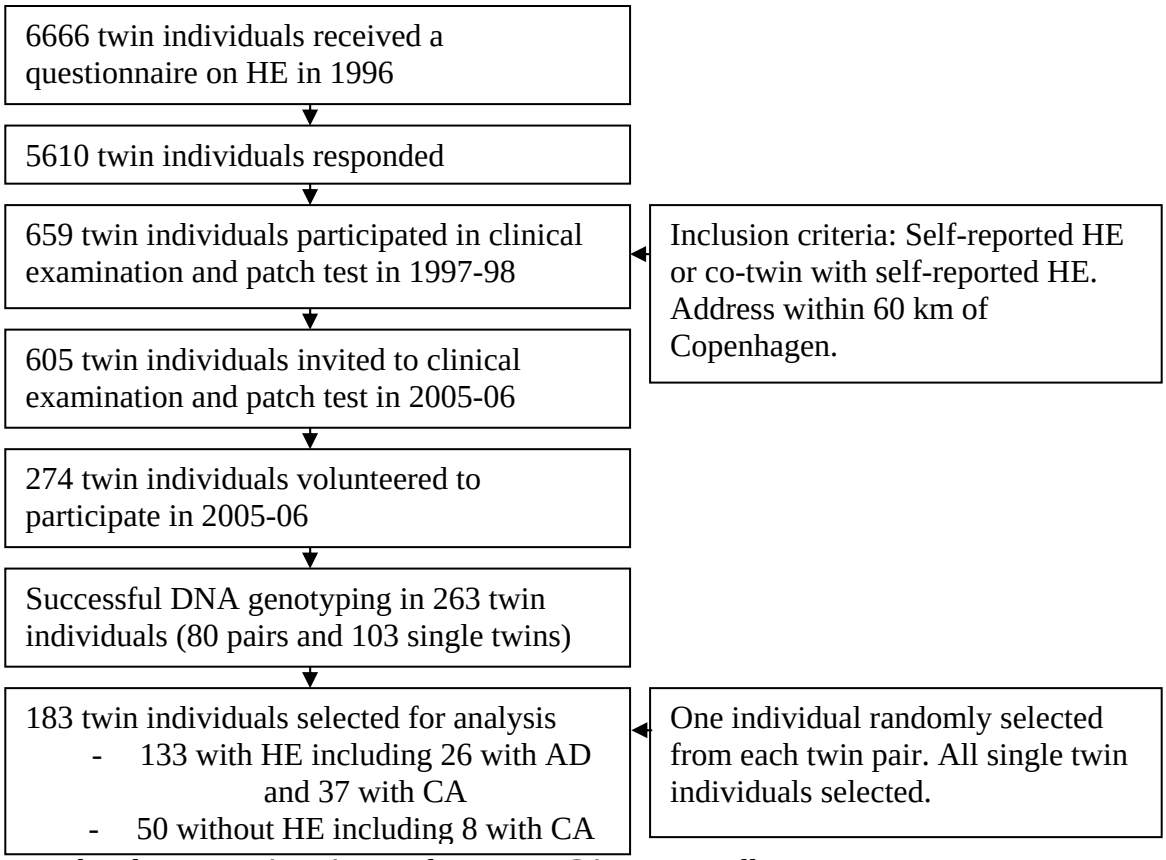
Figure 1. Flow-diagram illustrating recruitment of study population.



HE: hand eczema; AD: Atopic dermatitis; CA: contact allergy

Table 1. Frequency of filaggrin null alleles in the twin cohort and dependent on hand eczema, contact allergy and atopic dermatitis status as well as in the COPSAC cohort.

	All (N=183)	HE (N=133)	no HE (N=50)	CA (N=45)	no CA (N=136)	AD (N=26)	no AD (N=157)	HE and AD (N=26)	HE and no AD (N=107)	COPSAC (N=189)
Genotype					**R501X**					
AA	171 (93.4)	126 (94.7)	45 (90.0)	42 (93.3)	127 (93.4)	23 (88.5)	148 (94.3)	23 (88.5)	103 (96.3)	182 (96.3)
Aa	12 (6.6)	7 (5.3)	5 (10.0)	3 (6.7)	9 (6.6)	3 (11.5)	9 (5.7)	3 (11.5)	4 (3.7)	7 (3.7)
aa	0	0	0	0	0	0	0	0	0	0
					2282del4					
AA	171 (93.4)	123 (92.5)	48 (96.0)	41 (91.1)	128 (94.1)	23 (88.5)	148 (94.3)	23 (88.5)	100 (93.5)	180 (95.2)
Aa	12 (6.6)	10 (7.5)	2 (4.0)	4 (8.9)	8 (5.9)	3 (11.5)	9 (5.7)	3 (11.5)	7 (6.5)	9 (4.8)
aa	0	0	0	0	0	0	0	0	0	0
					Combined frequency					
AA	159 (86.9)	116 (87.2)	43 (86.0)	38 (84.4)	119 (87.5)	20 (76.9)	139 (88.5)	20 (76.9)	96 (89.7)	174 (92.1)
Aa	24 (13.1)	17 (12.8)	7 (14.0)	7 (15.6)	17 (12.5)	6 (23.1)	18 (11.5)	6 (23.1)	11 (10.3)	14 (7.4)
aa	0	0	0	0	0	0	0	0	0	1 (0.5)

Numbers in parentheses are percentages.
AA: homozygous genotype for *no* R501X / 2282del4.
Aa: heterozygous genotype for R501X / 2282del4.
aa: homozygous genotype for R501X / 2282del4.
HE: hand eczema; CA: contact allergy; AD: atopic dermatitis.
COPSAC: From the COPSAC study[17]. Children without atopic dermatitis.

Table 2. Comparison of allele frequencies in different subgroups with the χ2 test.

	χ^2	OR (95% C.I.)	P-value
Comparison of individuals within the twin group (N)			
Twins with hand eczema (133) vs twins without hand eczema (50)	0.047	0.9 (0.35-2.32)	0.828
Twins with contact allergy (45) vs twins without contact allergy (136)	0.274	1.3 (0.50-3.34)	0.600
Twins with atopic dermatitis (26) vs twins without atopic dermatitis (157)	2.640	2.3 (0.82-6.53)	0.104
Comparison of individuals in twin subgroups and individuals in COPSAC (N)			
Twins with hand eczema (133) vs COPSAC (189)	2.048	1.7 (0.82-3.54)	0.152
Twins with contact allergy (45) vs COPSAC (189)	2.477	2.1 (0.82-5.60)	0.116
Twins with atopic dermatitis (26) vs COPSAC (189)	5.945	3.5 (1.21-9.99)	0.015

95% C.I.: 95% confidence interval.
COPSAC: From the COPSAC study[17]. Children without atopic dermatitis.

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