Susceptibility to hand eczema in high risk occupations: Contribution of genetic and environmental factors

Visser, Maaike

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3.2 FILAGGRIN LOSS-OF-FUNCTION MUTATIONS AND ATOPIC DERMATITIS AS RISK FACTORS FOR HAND ECZEMA IN APPRENTICE NURSES: PART II OF A PROSPECTIVE COHORT STUDY

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ABSTRACT

Background /Objectives
Environmental exposure and personal susceptibility both contribute to development of hand eczema. In this study, we investigated the effect of loss-of-function mutations in the filaggrin gene (FLG), atopic dermatitis and wet work exposure on the development of hand eczema in apprentice nurses.

Methods
Dutch apprentice nurses were genotyped for the four most common FLG mutations; atopic dermatitis and hand eczema history were assessed by questionnaire. Exposure and hand eczema during traineeships were assessed with diary cards.

Results
The prevalence of hand eczema during traineeships was higher among subjects with a history of hand eczema reported at inclusion. Hand washing during traineeships and at home increased the risk of hand eczema. After adjustment for the effects of exposure and FLG mutations, an odds ratio of 2.5 (90% confidence interval 1.7 – 3.7) was found for a history of atopic dermatitis. In this study, an increased risk of hand eczema conferred by FLG mutations could not be shown, but subjects with concomitant FLG mutations and atopic dermatitis showed the highest risk of hand eczema during traineeships.

Conclusion
A history of atopic dermatitis, a history of hand eczema and wet work exposure were the most important factors increasing the risk of hand eczema during traineeships.
INTRODUCTION

Hand eczema (HE), as a manifestation of contact dermatitis of the hands, is one of the most common occupational diseases in industrialized countries, and may account for up to 90% of all occupational skin diseases \(^1\,^2\). Skin exposure to irritants is a risk in occupations such as nursing, hairdressing and metalworking; in these occupations, 1-year prevalence rates of HE of up to 30% have been reported \(^3\,^7\). Although exposure is a prerequisite for the development of occupational HE, some workers are more susceptible than others. The best-known and firmly established susceptibility factor for the development of occupational HE is a history of atopic dermatitis (AD). The increased risk of developing occupational HE for individuals with a history of AD has long been recognized \(^8\,^10\), and recent population studies reported up to fivefold increased risks \(^11\,^13\). One of the possible causes of the risk-enhancing effect of AD is an impaired skin barrier. Experimental studies have shown that the barrier function of the skin of patients with AD is reduced as compared with healthy controls, even in uninvolved skin areas \(^14\,^16\). The mechanisms that underlie reduced skin barrier in AD are not fully clear, but recent research suggests that the epidermal protein filaggrin might play an important role \(^17\,^18\). In the stratum corneum, filaggrin contributes to structural strength by aggregating the keratin filaments, and its breakdown products support hydration, pH balance, anti-bacterial defence and resistance to UV-radiation \(^18\,^19\). Several loss-of-function mutations have been identified in the filaggrin gene (FLG), resulting in reduced amounts or, in the case of homozygotes, in the absence of filaggrin in the skin. The summed prevalence of individuals who carry one or more of the most common FLG loss-of-function mutations in European populations is reported to be 7-10\% \(^17\,^20\,^23\). The impact of these mutations on skin barrier function has been shown in animal models \(^24\), in patients with ichthyosis or AD \(^14\,^25\) and in 3-month old infants with and without eczema \(^26\). FLG loss-of-function mutations are strongly associated with AD; 16-44\% of the individuals with moderate to severe AD carry one or more FLG mutations \(^20\,^22\,^27\,^29\). A recent meta-analysis revealed a more than 3-fold increased risk for developing AD in carriers of either one of the R501X or 2282del4 mutations \(^30\). Because filaggrin is important for the barrier function of the skin, it is plausible that FLG mutations as such can increase the risk for occupational HE. Indeed, recent case-control studies found an association between FLG mutations and occurrence of occupational HE \(^31\,^34\). In the aetiologial relationship between FLG mutations and occupational HE, AD can be both an intermediate factor (as FLG mutations increase the risk of AD) and a co-determinant independent from FLG. In the present study, we aimed to gain more insight into the relative contributions of both FLG mutations and AD to the aetiology of occupational HE.

Knowledge of susceptibility factors could contribute to a more targeted prevention of occupational HE. In some countries, a history of HE and a history of AD are used to identify persons at risk in jobs with high skin exposure; susceptible workers are offered extra preventive measures and attention by their occupational physician \(^25\,^36\). It has not yet been investigated whether the predictive value of susceptibility screening
can be increased by adding a genetic susceptibility marker such as FLG mutations. Interestingly, 40% of FLG mutation carriers do not develop AD. This subgroup will not be recognised as susceptible in current prevention programs. Another issue is that most of the studies that have explored the effect of FLG mutations on contact dermatitis have not accounted for the extent of environmental exposure. Therefore, the relative contributions of FLG mutations and a history of or current AD, taking exposure into consideration, are still to be elucidated. We performed a prospective cohort study among apprentice nurses, who provided a DNA sample by buccal swab, filled in a questionnaire concerning symptoms of AD and atopy, and were consecutively followed up for 1-3 years, with regular monitoring of symptoms of HE as well as exposure to ‘wet work’ as assessed by diary cards. The general characteristics of this cohort, the exposure to wet work during follow-up and the occurrence of HE have been described in Part I of this study. The present article describes the influence of FLG mutations, AD and exposure on the risk of HE in this cohort.

**METHODS**

**Subjects**

A detailed description of the study population and inclusion procedure is provided in Part I of this study. In short, apprentices were recruited from 15 different Dutch vocational schools that prepare students for a career in healthcare (nursing or caregiving). Students were eligible for participation if they had recently started a traineeship with a duration of at least 10 weeks, or were expecting to do so within the next few weeks. The only exclusion criterion was the presence of chronic inflammatory disease (e.g. psoriasis or rheumatoid arthritis). Approval was obtained from the Medical Ethical Committee of the Academic Medical Center, Amsterdam, The Netherlands.

**DNA sampling and genotyping**

The four most common FLG loss-of-function mutations in European populations were genotyped: R501X, 2282del4, R2447X and S3247X. Subjects provided a buccal swab sample (Geneticlab Diagnostic & Research, Pordenone, Italy; http://www.geneticlab.it), and DNA material was obtained from buccal mucosa cells. For each subject, two swabs were obtained, and 2 ml of lysis buffer (Puregene® Cell Lysis Solution, Gentra Systems, Minneapolis, MN, USA) was added to each swab to disrupt the cells and stabilize the DNA. Extraction and genotyping for FLG mutations R501X, R2447X and S3247X was performed by KBioscience (http://www.kbioscience.co.uk). Genotyping was performed with the KASP single-nucleotide polymorphism genotyping system, a homogeneous fluorescence resonance energy transfer (FRET)-based system, coupled with competitive allele specific polymerase chain reaction (PCR). Blind duplicates and Hardy-Weinberg equilibrium tests were used as quality control tests. R501X was genotyped by using the primer pair GAATGCCTGGAGCTGTCTCG (C-allele)
and CTGAATGCCTGGAGCTGTCTCA (T-allele) with the common allele primer GCACTGGAGGAAGACAGTCTC. R2447X was genotyped by using the primer pair GAGTGCCTGGAGCTGTCTCG (C-allele) and GAGTGCCTGGAGCTGTCTCA (T-allele) with the common allele primer GAGGAAGACAGGATCCACCACA. S3247X was genotyped by using the primer pair GTGTCTGGAGCCGCTGCT (C-allele) and GGTGTCTGGAGCCGCTGCTTT (A-allele) with the common primer CTCCAGAACCATCGTGGATCTGT. Genotyping for 2282del4 was performed by sizing a fluorescently labeled PCR fragment on an Applied Biosystems 3100 or 3730 DNA sequencer (Foster City, CA, USA) as described previously.

**Questionnaires**

At inclusion, participants filled in a questionnaire including items on eczema, rhinitis, conjunctivitis and asthma, allergies and/or symptoms following exposure to dust, animals, pollen, foods, metals and wool, present or past skin diseases, the presence of any other chronic disease, medication use, present or past skin symptoms on the hands or fingers, and exposure to wet work during previous jobs/apprenticeships, secondary jobs, and leisure or household activities. Atopy was defined as the presence of two or more of the following: symptoms following exposure to common allergens, rhinitis, conjunctivitis or asthma. AD was assessed according to a slightly modified version of the UK Working Party criteria ‘questions only’ definition, in which onset below 2 years of age was replaced by onset below 5 years of age as a proxy of ‘childhood dermatitis’.

At the end of the follow-up period, an email questionnaire was sent to all participants still in the study. This final questionnaire included items on symptoms experienced during follow-up, consultation of general practitioners or dermatologists, changes in hand hygiene behaviour, the use of protective hand cream, information on traineeships and side jobs, and smoking.

**Exposure and symptoms during practical training**

During their traineeships, the students had to keep count of the wet work activities that they performed during several shifts, using special diary cards as described in detail in Part I of this study. Skin symptoms on the hands were also recorded on the cards. If no cards had been returned near the end of the traineeship, students were contacted by email and/or telephone to retrieve information about the type of traineeship and possible symptoms retroactively.

Following the classification for screening for HE symptoms proposed by Vermeulen et al., HE was defined as the presence of at least one of the following combinations of symptoms: fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules, plus duration of > 3 days or recurrence (symptoms reported more than once). As these criteria were originally developed for identifying cases of HE in a working population, we were concerned that, by using this definition, we would miss early-stage symptoms that may progress into HE. Therefore, we also used a more
lenient definition, ‘mild HE’, defined as the presence of at least one of the following symptoms or combinations: redness and itch, redness and scaling, itch and scaling, fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules, all irrespective of duration or recurrence.

Without specification, HE refers to any episode of HE during follow-up. Some analyses comprise students without a history of HE in contrast to students with a history of HE. The latter refers to HE at any time before or at inclusion. A first episode of HE during follow-up in a student without a history of HE is equivalent to ‘incident HE’.

### Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics version 19 and Microsoft Excel™. In the subgroup analyses, FLG mutation carriers were compared with FLG wild-type individuals. No distinction was made between homozygous, compound heterozygous or heterozygous FLG mutation carriers, because the subgroup of homozygous or compound heterozygous carriers was too small for subgroup analysis to be performed. Because AD can be both an intermediate factor and an independent co-determinant in the aetiological relationship between FLG mutations and HE, we chose to use stratified analyses to study the effects on HE of FLG mutations and AD, each in the absence and in the presence of the other factor.

The relative risks (RRs) and confidence intervals (CIs) for the subgroup analyses of HE symptoms reported at inclusion were computed by using cross-tabulated results and applying the following formulas in Excel: $RR = \frac{a}{a+b}/\frac{c}{c+d}$, with $a$ and $c$ being the numbers of HE cases in the ‘exposed’ and ‘referent’ groups, respectively; and $90\% \ CI(RR) = \exp[\ln(RR) \pm 1.645\times SE[\ln(RR)]]$, in which $SE[\ln(RR)] = \sqrt{\frac{b}{a(a+b)} + \frac{d}{c(c+d)}}$. The 90% CI corresponds to one-sided testing with $p < 0.05$.

Analysis of the combined influence of susceptibility and exposure factors on the risk of HE during follow-up was performed with generalized linear mixed models in SPSS. HE is a disease with a fluctuating course, and the recovery time may be as short as a few days. Thus, the apprentice nurses would have time to recover from HE in between traineeship periods. We therefore assumed that the probability to develop HE in one traineeship does not depend on the extent of exposure or on having had HE in previous traineeships. Each traineeship was therefore counted as a separate entity, and data from subjects who entered a second or a third traineeship were entered as multiple records in the database. This, however, results in the problem that, regardless of susceptibility, subjects who contributed data for multiple traineeships would have had more opportunities to develop HE than those who had been followed for only one traineeship. Therefore, a mixed models design was used in the analyses, with participant ID included as random effect (procedure GENLINMIXED in SPSS™). In such a mixed model, the within-subject correlation is taken into account.

Analysis of wet work exposure in this cohort had revealed that a frequency of hand washing during practical trainings > 8 times per shift, hand washing at home > 10 times per day and working in a side job involving wet work for > 8 hr a week increased
the risk of HE (Part I)\textsuperscript{42}. Therefore, these were included as binary variables in the multivariate mixed models to represent wet work exposure. Preceding this analysis, the mean frequency of hand washing in different healthcare sectors was assessed by linear mixed models with healthcare sector as fixed effect and subject ID as random effect (procedure MIXED in SPSS\textsuperscript{TM}). The mean frequency of hand washing during traineeships was lowest in psychiatry (7.0 times per shift), medium to high in homecare and hospitals (8.8 and 8.9 times per shift, respectively), and highest in care for the disabled and nursing homes (10.4 and 10.5 times per shift, respectively). The frequency of hand washing during traineeships was classified according to whether the traineeship was performed in psychiatry or in any other sector, which corresponds to a cut-off value of (supposedly) 8 times per shift. This classification was applied to all subjects.

Use of hand cream, exposure to wet work and number of subjects reporting HE during traineeships (Fig. 2) was compared between the four subgroups categorized by FLG and AD by use of the chi-square test.

RESULTS
Study population
The participation rate of the apprentices invited was ~50%. A total of 728 apprentice nurses completed the inclusion questionnaire. Seven apprentices were excluded because of chronic inflammatory disease. Some participants did not provide a buccal swab sample at inclusion, because they were aged < 18 years (in The Netherlands, these persons are only allowed to provide a DNA-sample with parental consent) and DNA sampling was postponed until parental consent was obtained or until they had turned 18 years during follow-up. Eventually, a total of 626 DNA samples were obtained, 596 of which were successfully genotyped for all four investigated FLG loss-of-function mutations (R501X, 2282del4, R2447X and S3247X). A further 150 participants were lost to follow-up shortly after completion of the inclusion questionnaire or quit the study before going through a traineeship (mostly because of changing career), leaving a total of 446 participants in whom to study the impact of susceptibility factors on the risk of developing HE during vocational training.

Genotype distributions and associations with atopic disease
Table 1 shows the genotype distributions. FLG mutations were present in 11.1% of the participants. Fifty-six individuals were heterozygous for one mutation, 2 were homozygous for 2282del4, 3 were homozygous for R2447X and 1 was compound heterozygous for 2282del4 and R501X.

The genotype distributions were not in Hardy Weinberg-equilibrium for 2282del4, R2447X, and the combined genotype. This was probably because of the relatively large number of homozygotes among subjects with AD, combined with a slight overrepresentation of subjects with AD in this cohort (see Part I)\textsuperscript{42}. In participants
without AD (n=460), the genotype distributions for 2282del4 and the combined genotype were in Hardy Weinberg-equilibrium.

FLG mutations were associated with a history of AD (RR 1.8; 90% CI 1.37 – 2.35), especially with persistent AD starting before 5 years of age and still present at the time of inclusion (RR 2.6; 90% CI 1.43 – 4.67). There were also associations between FLG mutations and general dry skin (RR 2.5; 90% CI 1.82 – 3.38) and between FLG mutations and symptoms upon exposure to common allergens (RR 1.3; 90% CI 1.01 – 1.54). No association was found with rhinitis or asthma.

**Table 1.** Genotype distributions for the filaggrin gene (FLG) loss-of-function mutations R501X, 2282del4, R2447X and S3247X in apprentice nurses

<table>
<thead>
<tr>
<th>FLG mutation</th>
<th>R501X</th>
<th>2282del4</th>
<th>R2447X</th>
<th>S3247X</th>
<th>Combined (four mutations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group size</td>
<td>608</td>
<td>614</td>
<td>610</td>
<td>607</td>
<td>596</td>
</tr>
<tr>
<td>AA, n (%)</td>
<td>587</td>
<td>576</td>
<td>604</td>
<td>605</td>
<td>530</td>
</tr>
<tr>
<td></td>
<td>(96.5)</td>
<td>(93.8)</td>
<td>(99.0)</td>
<td>(99.7)</td>
<td>(88.9)</td>
</tr>
<tr>
<td>Aa, n (%)</td>
<td>21</td>
<td>35</td>
<td>3</td>
<td>2</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>(3.5)</td>
<td>(5.7)</td>
<td>(0.5)</td>
<td>(0.3)</td>
<td>(9.9)</td>
</tr>
<tr>
<td>aa, n (%)</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(0)</td>
<td>(0.5)</td>
<td>(0.5)</td>
<td>(0)</td>
<td>(1.2)</td>
</tr>
<tr>
<td>Total FLG carriers</td>
<td>21</td>
<td>38</td>
<td>6</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>(Aa + aa), n(%)</td>
<td>(3.5)</td>
<td>(6.2)</td>
<td>(1.0)</td>
<td>(0.3)</td>
<td>(11.1)</td>
</tr>
<tr>
<td>Wild-type allele frequency (%)</td>
<td>98.3</td>
<td>96.7</td>
<td>99.3</td>
<td>99.8</td>
<td>93.9</td>
</tr>
<tr>
<td>Mutant allele frequency (%)</td>
<td>1.7</td>
<td>3.3</td>
<td>0.7</td>
<td>0.2</td>
<td>6.1</td>
</tr>
</tbody>
</table>

**Symptoms of HE reported at inclusion**

We used stratified analyses to investigate the effect of FLG mutations and AD on the occurrence of HE. The study population was divided into four groups: (i) participants without FLG mutations and with no history of AD (FLG-/AD-); (ii) participants with FLG mutations but with no history of AD (FLG+/AD-); (iii) participants without FLG mutations but with a history of AD (FLG-/AD+); and (iv) participants with both FLG mutations and a history of AD (FLG+/AD+). In a retrospective approach, we compared past and present symptoms of HE reported in the inclusion questionnaire between these four groups (Table 2). In total, 54% of all participants reported one or more skin symptoms, 13% had a history of HE, and 7% had HE at the time of inclusion. Regardless of FLG mutations, a history of AD conferred an increased RR for all investigated symptoms. Participants with concomitant FLG mutations and AD (FLG+/AD+) showed the highest symptom prevalence, and a significantly higher prevalence of scaling, fissures and current HE than the FLG-/AD+ subgroup. Among subjects without a history of AD, those who carried one or more FLG mutations (FLG+/AD-) did
not report more symptoms on the hands and fingers or HE before or at inclusion than those without FLG mutations.

Of the 7 homozygous or compound heterozygous apprentices among the FLG mutation carriers, 6 had a history of AD, 3 had HE at the time of inclusion, and another 3 reported a history of HE.

**HE during traineeships: effects of AD, FLG mutations, and wet work**

One to three years of follow-up was completed for 446 participants. One hundred and thirty participants (29%) reported HE on one or more occasions during their traineeships. Three hundred and fifty-nine subjects (81%) had no HE history up to the time of inclusion. Of these, 78 (22%) developed HE during their traineeships. Among the participants with a history of HE but no HE at the time of inclusion (n=52), 29 (56%) reported HE during one or more traineeships. Thirty-five subjects (8%) had HE at the time of inclusion. Mixed models analysis showed that, after adjustment for the effects of exposure, participants with a history of HE up to inclusion were at increased risk of developing HE during traineeships [odds ratio (OR) 4.5; 90% CI 2.96 – 6.98]. After taking into account AD history, the OR for having HE during traineeships for participants with a history of HE was 3.9 (90% CI 2.5 – 6.1). Twenty participants who had HE at the time of inclusion also reported HE during their first traineeship. Because, for these participants, it was unknown whether their HE was related to their traineeship or was a continuation of already existing HE, a second analysis was performed excluding

**Fig. 1.** Reported period prevalence of hand eczema (HE) during traineeships in the first and second year of follow-up in participants with or without a history of HE reported at inclusion.
Table 2. Number of apprentice nurses reporting having (or having had) symptoms of hand eczema at inclusion, and relative risk (RR) ratios for symptoms of hand eczema in four subgroups based on filaggrin gene (FLG) mutations and history of atopic dermatitis (AD).

<table>
<thead>
<tr>
<th>Subgroup name:</th>
<th>FLG-/AD-</th>
<th>FLG+/AD-</th>
<th>FLG-/AD+</th>
<th>FLG+/AD+</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLG mutations*</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>History of ADb</td>
<td>N = 405</td>
<td>N = 38</td>
<td>N = 125</td>
<td>N = 28</td>
</tr>
<tr>
<td>Reported symptoms on the hands/ fingers at inclusion:</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Redness</td>
<td>84 (21)</td>
<td>7 (18)</td>
<td>60 (48)</td>
<td>16 (57)</td>
</tr>
<tr>
<td>Scaling</td>
<td>88 (22)</td>
<td>5 (13)</td>
<td>47 (38)</td>
<td>15 (54)</td>
</tr>
<tr>
<td>Itch</td>
<td>145 (36)</td>
<td>14 (37)</td>
<td>74 (59)</td>
<td>18 (64)</td>
</tr>
<tr>
<td>Fissures</td>
<td>91 (22)</td>
<td>11 (29)</td>
<td>46 (37)</td>
<td>15 (54)</td>
</tr>
<tr>
<td>Vesicles</td>
<td>48 (12)</td>
<td>0 (0)</td>
<td>32 (26)</td>
<td>8 (29)</td>
</tr>
<tr>
<td>Bumps</td>
<td>74 (18)</td>
<td>2 (5)</td>
<td>46 (37)</td>
<td>9 (32)</td>
</tr>
<tr>
<td>History of mild hand eczema c</td>
<td>163 (40)</td>
<td>13 (34)</td>
<td>81 (65)</td>
<td>19 (68)</td>
</tr>
<tr>
<td>History of hand eczema d</td>
<td>54 (13)</td>
<td>2 (5)</td>
<td>37 (30)</td>
<td>12 (43)</td>
</tr>
<tr>
<td>Mild hand eczema currently present</td>
<td>58 (14)</td>
<td>7 (18)</td>
<td>32 (26)</td>
<td>9 (32)</td>
</tr>
<tr>
<td>Hand eczema currently present</td>
<td>18 (4)</td>
<td>1 (3)</td>
<td>14 (11)</td>
<td>7 (25)</td>
</tr>
</tbody>
</table>

CI, confidence interval
* Carrier of one or more of the following loss-of-function mutations in FLG: R501X, 2282del4, R2447X or S3247X.

b A history of atopic dermatitis was assessed by questionnaire, with a slightly modified version of the UK Working Party criteria.

c One or more of the following combinations of symptoms: redness and itch, redness and scaling, scaling and itch, fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules.

d One or more of the following combinations of symptoms: fissures and redness, fissures and itch, fissures and scaling, vesicles, or papules, plus duration of >3 days or recurrence (symptoms reported more than once).

* Significant at α < 0.05.

these participants, which resulted in an OR of 2.9 (90% CI 1.80 – 4.70). Fig. 1. shows the prevalence of HE in subjects with or without a history of HE reported at inclusion, divided into first and recurrent episodes of HE; it shows the high prevalence of HE during traineeships among participants with a history of HE at inclusion and the high recurrence rate for this group.
Table 2. Number of apprentice nurses reporting having (or having had) symptoms of hand eczema at inclusion, and relative risk (RR) ratios for symptoms of hand eczema in four subgroups based on filaggrin gene (FLG) mutations and history of atopic dermatitis (AD).

<table>
<thead>
<tr>
<th>Subgroup name</th>
<th>FLG-/AD-</th>
<th>FLG-/AD+</th>
<th>FLG+/AD+</th>
<th>FLG+/AD+</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLG-/-ad-</td>
<td>0.9</td>
<td>2.3*</td>
<td>2.8*</td>
<td>3.1*</td>
</tr>
<tr>
<td>(0.50 – 1.59)</td>
<td>(1.85 – 2.89)</td>
<td>(2.01 – 3.77)</td>
<td>(1.48 – 6.51)</td>
<td>(0.87 – 1.62)</td>
</tr>
<tr>
<td>FLG+/ad-</td>
<td>0.6</td>
<td>1.7*</td>
<td>2.5*</td>
<td>4.1*</td>
</tr>
<tr>
<td>(0.30 – 1.22)</td>
<td>(1.35 – 2.21)</td>
<td>(1.78 – 3.42)</td>
<td>(1.68 – 9.88)</td>
<td>(1.01 – 2.01)</td>
</tr>
<tr>
<td>FLG-/ad+</td>
<td>1.0</td>
<td>1.7*</td>
<td>1.8*</td>
<td>1.7*</td>
</tr>
<tr>
<td>(0.71 – 1.48)</td>
<td>(1.40 – 1.95)</td>
<td>(1.39 – 2.32)</td>
<td>(1.06 – 2.88)</td>
<td>(0.84 – 1.41)</td>
</tr>
<tr>
<td>FLG+/ad+</td>
<td>1.3</td>
<td>1.6*</td>
<td>2.4*</td>
<td>1.9*</td>
</tr>
<tr>
<td>(0.83 – 2.01)</td>
<td>(1.28 – 2.09)</td>
<td>(1.72 – 3.31)</td>
<td>(1.01 – 3.39)</td>
<td>(1.03 – 2.06)</td>
</tr>
<tr>
<td>n.a.</td>
<td>0.3*</td>
<td>2.2*</td>
<td>2.4*</td>
<td>n.a.</td>
</tr>
<tr>
<td>(1.54 – 3.02)</td>
<td>(1.41 – 4.14)</td>
<td>n.a.</td>
<td>(0.64 – 1.94)</td>
<td></td>
</tr>
<tr>
<td>RR (90%CI)</td>
<td>0.9</td>
<td>2.0*</td>
<td>1.8*</td>
<td>6.1*</td>
</tr>
<tr>
<td>(0.09 – 0.91)</td>
<td>(1.55 – 2.61)</td>
<td>(1.08 – 2.85)</td>
<td>(1.43 – 26.10)</td>
<td>(0.53 – 1.43)</td>
</tr>
<tr>
<td>FLG-/AD-</td>
<td>0.9</td>
<td>1.6*</td>
<td>1.7*</td>
<td>2.0*</td>
</tr>
<tr>
<td>(0.58 – 1.25)</td>
<td>(1.39 – 1.87)</td>
<td>(1.33 – 2.13)</td>
<td>(1.19 – 3.30)</td>
<td>(0.82 – 1.33)</td>
</tr>
<tr>
<td>FLG-/AD-</td>
<td>0.4</td>
<td>2.2*</td>
<td>3.2*</td>
<td>8.1*</td>
</tr>
<tr>
<td>(0.12 – 1.25)</td>
<td>(1.63 – 3.02)</td>
<td>(2.12 – 4.87)</td>
<td>(1.98 – 33.52)</td>
<td>(0.95 – 2.21)</td>
</tr>
<tr>
<td>FLG+/AD-</td>
<td>1.3</td>
<td>1.8*</td>
<td>2.2*</td>
<td>1.7</td>
</tr>
<tr>
<td>(0.71 – 2.33)</td>
<td>(1.30 – 2.46)</td>
<td>(1.37 – 3.68)</td>
<td>(0.74 – 4.12)</td>
<td>(0.75 – 2.10)</td>
</tr>
<tr>
<td>FLG+/AD-</td>
<td>0.6</td>
<td>2.5*</td>
<td>5.6*</td>
<td>9.5*</td>
</tr>
<tr>
<td>(0.11 – 3.14)</td>
<td>(1.44 – 4.42)</td>
<td>(2.91 – 10.87)</td>
<td>(1.24 – 72.89)</td>
<td>(1.13 – 4.40)</td>
</tr>
</tbody>
</table>

* Significant at $\alpha < 0.05$.  

The prevalence of HE during the first and second traineeship in the four subgroups of participants with or without AD and FLG mutations is shown in Fig. 2. Increased prevalence rates of HE were seen for the FLG-/AD+ subgroup and the FLG+/AD+ subgroup as compared with the FLG-/AD- control group. Both Figs. 1 and 2 are restricted to the first and second traineeships, because the number of participants...
that had completed a third traineeship was too small (n=57) for subgroup analysis to be performed.

The exposure did not differ appreciably between the four subgroups; the proportion of individuals who had traineeships in healthcare sectors with frequent hand washing (cut-off at > 8 times per shift) ranged from 63% to 71%.

The effects of AD, FLG mutations and exposure on the risk of developing HE during traineeships were calculated using a mixed models design. On the basis of the results of Part I of this study, the frequency of hand washing during traineeships, hand washing at home > 10 times a day and working in a side job involving wet work (e.g. healthcare, bars, or restaurants) were included in the models to account for wet work exposure. A first crude analysis resulted in an unadjusted OR of 1.1 (90% CI 0.7 – 2.0) for FLG mutations and an unadjusted OR of 2.8 (90% CI 1.9 – 4.1) for AD.

Table 3 shows the results of two multivariate mixed models including the four susceptibility subgroups together with exposure; in both models, the occurrence of (mild) HE in FLG wild-type participants without AD serves as the reference. Model 1 shows that, after adjustment for the effects of exposure, a history of AD and the combination of a history of AD and FLG mutations increased the risk of HE during vocational training with ORs, respectively, of 2.2 and 3.6. Taking into account the group sizes, the weighted OR of AD was 2.5 (90% CI 1.7 – 3.7). For mild HE, the corresponding ORs were both 2.1. For FLG mutations in participants without a history of AD, no effect could be shown (OR 0.7). Frequent hand washing during traineeships

Fig. 2. Reported period prevalence of hand eczema (HE) during the first and second traineeships in four subgroups of participants. * p < 0.05, *** p < 0.0001.
 (> 8 times per shift) and frequent hand washing at home (> 10 times per day) increased the risk of hand eczema, with ORs of 2.2 and 1.8, respectively.

Model 2 concerns HE during traineeships in participants who had been free from HE up to inclusion, and who had not been exposed to skin irritants before entering the study (e.g. previous education or career involving wet work or traineeships in previous school years, because the extent of that exposure could not be estimated). A similar tendency for an increased risk of HE was found for AD in combination with FLG mutations and for frequent hand washing at home.

As these results suggest that the influence of FLG mutations on HE differs between participants with and without AD, we investigated the existence of interaction in a model including FLG, AD, an interaction term between FLG and AD, and exposure. In the model including all participants (Model 1), no significant interaction effect could be shown [OR(interaction) 2.1; 90% CI 0.6 – 7.1]. In the model including only participants without previous HE or exposure (Model 2), a tendency (p = 0.08) for interaction was found [OR(interaction) 5.4; 90% CI 1.1 – 25.9].

**Use of hand cream**

Use of hand cream at least once a day was reported by 53% of the subjects in the FLG-/AD- subgroup. The usage of hand cream was significantly more frequent than this in the FLG-/AD+ subgroup (68%, chi-square, p = 0.04) and in the FLG+/AD+ subgroup (90%, p = 0.006), but not in the FLG+/AD- subgroup (62%, p = 0.190).

**DISCUSSION**

This study examined both genetic susceptibility and environmental exposure as risk factors for HE. Regarding AD, we found a distinct effect on HE with both the follow-up and the retrospective approaches. During follow-up, we found no indication of an increased risk of HE conferred by FLG loss-of-function mutations, although the apprentices with FLG mutations in addition to a history of AD had the highest OR of HE during traineeships (OR 3.6). With the retrospective approach, FLG mutations only had an effect on HE at inclusion in participants with AD. Frequent hand washing during traineeships (> 8 times per shift) or at home (> 10 times per day) increased the risk of HE during follow-up, with ORs of 2.2 and 1.8, respectively.

Our results confirm that a history of AD is associated with an increased risk of HE in high risk occupations, a finding that has been made in several epidemiological studies.45-49 The fact that we could not show an effect of FLG mutations in the present study was unexpected. The high OR for HE during traineeships among participants with concomitant FLG mutations and AD is consistent with the results reported in our recent case-control study on occupational contact dermatitis patients and vocational students in training for high risk occupations. In that study, an effect of FLG mutations...
irrespective of AD (OR 1.6; 95% CI 1.0 – 2.6) was found 34. The cases in that study, however, had chronic and severe HE, as opposed to the apprentice nurses, who often had less severe HE, which might not become chronic. Possibly, higher exposure or a longer duration is needed to reveal an effect of FLG mutations.

In a recent cross-sectional population study, Thyssen et al. found that FLG mutations constituted a risk factor for HE in individuals with AD, but not in individuals without AD 33. Our data also point to an interactive effect, although this was only indicated in part of the analyses (Model 2).

The absence of a significant effect of FLG mutations in this study could not be explained by differences in exposure or use of hand cream. A possible explanation
might be that some FLG mutation carriers are able to compensate reduced amounts of filaggrin in their skin via an as yet unknown mechanism, preventing them from developing AD as well as HE. This may partly explain the wide range in susceptibility to HE (OR 0.7 – OR 3.6) that we observed among FLG mutation carriers, which – in view of the observed tendency for interaction – is partly related to the absence or presence of AD. More research into skin barrier properties of FLG mutation carriers without AD may shed more light on this.

Irrespective of FLG mutations, a possible role of the filaggrin protein itself may be considered. Recently, one study investigated skin lipid composition, irritation response and the skin barrier in AD patients and controls, both with and without FLG mutations. No difference in stratum corneum lipid composition or increase in TEWL after a 24-h irritation test was found between FLG mutation carriers and FLG wild-type individuals. Another recent study also found no difference in lipid composition and skin barrier function between AD patients with and without FLG mutations. However, there was a significant positive correlation between favourable lipid organization and skin barrier function with natural moisturizing factors (NMF) in the stratum corneum. As NMF can be seen as a proxy for filaggrin expression, this might imply that filaggrin itself does play a role in the stratum corneum lipid composition and skin barrier function. Indeed, research among ichthyosis vulgaris patients carrying FLG mutations showed that filaggrin deficiency led to a paracellular defect in skin barrier function, caused by disrupted lipid bilayer organization and altered loading of lamellar bodies. In addition to the loss-of-function mutations, several other factors, mostly associated with AD, can influence filaggrin levels in the skin. For instance, the expression of filaggrin may be downregulated by inflammatory cytokines, for example interleukin (IL)-4, IL-13, IL-22 and IL-25. Also, Brown et al. have recently shown that the number of filaggrin repeats in the FLG gene may vary between 10, 11 or 12, and that these copy number variations are significantly associated with the risk of AD. It might be speculated that variation in filaggrin expression caused by copy number variations may also play a role in susceptibility to occupational HE. Future studies investigating the role of filaggrin in occupational HE should consider the inclusion of copy number variations in their analysis.

Some limitations of this study need to be mentioned. First, because of the multiple traineeships in which participants were repeatedly at risk of HE, a mixed model analysis was used. We note that the ORs obtained from the models are an overestimation of the RRs. This especially applies to subgroups with a high prevalence of HE. Corresponding RRs can be calculated by using the estimated means obtained from the models. For example, in Model 1, the ORs for HE of 0.7, 2.2 and 3.6 for the FLG+/AD-, FLG-/AD+ and FLG+/AD+ subgroups correspond to RRs of, respectively, 0.7, 1.9 and 2.5.

Second, detailed information on wet work exposure was available for only 383 of the 446 participants who were followed-up in this cohort. Repeating the mixed models analysis in this subset of participants yielded similar results as when all subjects were included, which justifies the use of extrapolated exposure variables.
A third limitation is that, on the basis of the symptoms as reported, we were not able to distinguish between HE of the irritant, allergic or atopic type. Despite a similar clinical appearance, these subtypes of HE have different underlying mechanisms, and are probably not equally affected by genetic susceptibility factors. For example, FLG loss-of-function mutations have shown positive associations with irritant, but less so with allergic contact dermatitis. Patch testing would be needed to differentiate between participants with irritant HE and those with allergic HE, but this was not feasible in our study. Among the 52 participants with reported symptoms of HE who were seen by the collaborating occupational physician, contact allergy was diagnosed in, at most, 23% (Part I). If we had been able to exclude the cases with contact allergy from our study, this would probably have shifted the ORs for HE resulting from FLG mutations a little away from 1. Also, we were not able to assess severity of HE on the basis of the self-reported symptoms. The use of hand cream by 90% of the participants in the FLG+/AD+ subgroup, however, suggests more severe HE in this subgroup. Possibly, a stronger effect of FLG mutations would have been found in association with severity of HE.

One of the underlying reasons for this study was to investigate whether adding FLG genotyping to the AD screening tool would improve the identification of susceptible individuals in high risk occupations. Our results do not convincingly indicate that this is the case. Even if the effect of FLG in subjects with a history of AD had been significant, the effect size would probably be too small for a substantial favourable effect on the predictive values of a screening procedure. Information about AD and HE history, as is currently asked for according to Dutch and German guidelines, is a feasible predictor the acquisition of occupational HE, as our results have confirmed. The results of our case-control study and our present prospective cohort study show that those in the FLG+/AD+ subgroup are at the highest risk for occupational HE. Furthermore, occupational HE patients with concomitant AD and FLG mutations appeared to have a worse prognosis than FLG-/AD-, FLG-/AD+ or FLG+/AD- patients in a recent follow-up study. New research might confirm that AD patients with FLG mutations are indeed substantially more susceptible to occupational HE than patients with AD without FLG mutations. If this is so, identifying FLG mutations among AD patients and advising avoidance of irritant exposure in such patients would be beneficial.

CONCLUSION AND RECOMMENDATIONS
A history of AD, a history of HE and wet work exposure were the most important factors increasing the risk of HE during traineeships. As our results confirmed that HE develops shortly after the start of exposure to wet work, even in traineeships, it is strongly recommended to start prevention programs as early as during vocational training, instead of at the time of employment. In addition, it would be interesting to further investigate skin barrier properties of FLG mutation carriers without AD, which may shed more light on the existence of possible mechanisms to compensate for reduced filaggrin in the skin.
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