Individual susceptibility to chronic irritant contact dermatitis

de Jongh, C.M.

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Chapter 4.3

Loss-of-function polymorphisms in the filaggrin gene and susceptibility to chronic irritant contact dermatitis

C.M. de Jongh¹, M.M. Verberk¹, F. Calkoen¹, F.J.H. van Dijk¹, S.M. John², L. Khrenova², S. Kežić¹

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¹Coronel Institute of Occupational Health, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; ²Department of Dermatology, Environmental Medicine and Health Theory, University of Osnabrueck, Osnabrueck, Germany
Abstract

Background and objectives Polymorphisms in the filaggrin (FLG) gene which result in loss of filaggrin production, may alter the skin barrier and are a well-known predisposing factor for atopic dermatitis. As a compromised skin barrier and atopic dermatitis are risk factors for chronic irritant contact dermatitis (CICD), our objective was to determine whether polymorphisms in the FLG gene contribute to the occurrence of occupational CICD.

Methods In a case-control study, the FLG polymorphisms R501X and 2282del4 were determined in 206 patients with CICD. 217 apprentices in vocational training for high-risk occupations for CICD were chosen as controls. Data on skin condition were collected by dermatologists for patients and questionnaires for controls.

Results We found no clear difference between the patient and control group in the frequency of carriers of a FLG null allele (9.7% vs. 7.4%; OR = 1.35, 95%-CI 0.68 - 2.69). The frequency of carriers in CICD patients with skin atopy (11.9%) tended to be higher than in CICD patients without atopy (3.6%; P = 0.08), but not higher compared with the control group (7.4%; P = 0.14). The lifetime prevalence of flexural eczema was higher in carriers compared with non-carriers in the CICD patient population (60% vs. 32%; P = 0.014), but less clearly in controls (36% vs. 19%; P = 0.13).

Conclusions Our study suggests that FLG polymorphisms are not a substantial risk factor of CICD. The increased risk of FLG null allele-carriers on flexural eczema, a characteristic of atopic dermatitis, is in line with previous findings.
Introduction

Workers involved in wet work or having frequent skin contact with chemicals, for example hairdressers, nurses, metalworkers and cleaners are at high risk to acquire chronic irritant contact dermatitis (CICD). This skin disease, which is most frequently localized on the hands, is characterized by dryness, redness, scaling and hyperkeratosis. Occupationally induced CICD often has a poor prognosis and may lead to long sick leave periods, change of occupation and an impaired quality of life. CICD is a multifactorial disorder; both genetic and environmental factors play a role in its development. However, the mechanism of the development of CICD and factors that predispose individuals to this skin disease are only partially understood.

The stratum corneum (SC) constitutes the principal barrier of the skin to penetration of substances. A proper skin barrier function is essential in the prevention of skin irritation when working with irritants. The protein filaggrin plays an important role in the structure and the hydration of the SC. Filaggrin, acronym for filament-aggregating protein, promotes the flattened shape of corneocytes by packing keratin filaments into bundles to form the keratin skeleton. During the cornification process, the protein profilaggrin is proteolytically cleaved and released as 10 to 12 individual filaggrin units. After exerting its role in the formation of keratin filament bundles, filaggrin is degraded, mostly into free amino acids. These amino acids, which form the majority of the natural moisturising factors, are highly hygroscopic and contribute to the water retention in the SC. The gene encoding filaggrin (FLG) is located within the epidermal differentiation complex on chromosome 1q21, a dense cluster of genes involved in the terminal epidermal differentiation and formation of the SC. Recently, two polymorphisms in the FLG gene were identified, which both encode for a premature termination codon located in the first FLG repeat, leading to a complete loss of processed filaggrin. These mutations were originally found to be a cause of ichthyosis vulgaris, a keratinisation disorder characterised by a very dry and flaky skin. Processed filaggrin was completely absent in homozygous carriers and compound heterozygous carriers, i.e. heterozygous for both polymorphisms. Upon further investigation, these so called FLG null alleles, designated as R501X and 2822del4, were also shown to be a predisposing factor for atopic dermatitis. Atopic dermatitis is a chronic inflammatory skin disease characterized by pruritic, eczematoid lesions and a course marked by exacerbations and remissions. The symptoms usually start in early infancy or childhood, often with flexural eczema, and can persist into adulthood. Several
studies showed that, depending on the disease phenotype, between 16% and 56% of atopic dermatitis patients carried one or more \textit{FLG} null alleles, in comparison with 5% to 10% of subjects in the general European population.\textsuperscript{13,15-21} These findings confirm the already existing hypothesis of a barrier dysfunction underlying atopic dermatitis in at least part of the patients.\textsuperscript{22}

Using immunohistochemistry, heterozygote carriers of a \textit{FLG} null allele were found to have reduced amounts of filaggrin in their epidermis compared to subjects with the wildtype genotype.\textsuperscript{23,24} In line, heterozygotes had a significant lower amount of natural moisturizing factor in their SC as determined by confocal Raman spectroscopy.\textsuperscript{25} In addition, these carriers had a higher baseline transepidermal water loss (TEWL) and an increased skin irritability to sodium lauryl sulphate (manuscript in preparation). Despite of an impaired skin barrier associated with a filaggrin deficiency, not all persons carrying a \textit{FLG} null allele had a history of atopic dermatitis.\textsuperscript{13,25}

Present or past atopic dermatitis is a predisposing factor for hand eczema corresponding with a factor 2 increased risk\textsuperscript{26-28} and for CICD.\textsuperscript{29} As \textit{FLG} polymorphisms appeared to be a substantial risk factor for atopic dermatitis, an elevated frequency of these polymorphisms is to be expected in the CICD patients with a history of atopic dermatitis. The hypothetic role of \textit{FLG} null alleles in the causation of CICD among those with atopic dermatitis is shown as pathway A in Figure 1. In addition, as a filaggrin deficiency affects the skin barrier, this may also directly increase an individual’s susceptibility to skin irritants and induce CICD following pathway B (Figure 1). Skin exposure to irritants is needed to develop CICD, but may also play a role in the development of atopic dermatitis.
Our objective was to determine whether the FLG R501X and 2282del4 polymorphisms contribute to the occurrence of CICD. In a case-control study, the frequencies of these polymorphisms in a group of 206 patients with occupational CICD were compared with the data of 217 control subjects. We selected a control group which represents the genetic distribution of the source population from which the patients originate. So we chose as controls second and third year students in vocational training for high-risk occupations for CICD. Further, based on data obtained by the dermatologists involved, we investigated the association of the FLG null alleles with the sub-diagnoses, type IV sensitizations, dry skin, flexural eczema and time of onset of hand eczema in CICD patients. In the control group, we investigated the association between these FLG null alleles and self-reported skin abnormalities at the hands and flexural eczema.

Methods

Study population
The inclusion criterion for the patients was CICD of the hands or forearms at examination during ≥ 3 months, or medically verified CICD of the hands or forearms during at least 1 period of ≥ 3 months in the past. All consecutive patients who were seen in the Department of Dermatology, University of Osnabrueck between November 2005 and April 2007 and met the
inclusion criteria were invited to participate; only 16 refused for personal reasons. 206 patients were included in the study. All patients visit this clinic, which is specialised for occupational dermatology, by virtue of the statutory employers' accident liability insurances. Most of them were referred by their local dermatologists. Further, 75% are in-patients, who participate in a specific interdisciplinary treatment and prevention programme, and 25% are outpatients.\textsuperscript{30} For each patient, a dermatologist completed a detailed checklist on sub-diagnoses, course of the disease, present or past flexural eczema, other atopic signs and on occupational activities. The patients were classified using the following three diagnoses regarding the hands (i) CICD without atopy, i.e. erythema, scaling and fissures, located at the back of the hands or the interdigital web spaces, strictly exposure-dependent course and no signs of atopy, (ii) CICD with atopic skin disposition, i.e. clinical features as (i), but additionally previous atopic dermatitis (including flexural eczema) or a combination of so called ‘minor criteria’ of skin atopy,\textsuperscript{31-33} and (iii) irritant-induced atopic eczema, i.e. predominantly palmar itch, vesicles, erythema, oozing, symmetrically present and protracted course after exposure discontinuation.\textsuperscript{34,35} The patients were employed in varying job sectors at the start of their hand eczema: (I) health care sector, (II) metal and construction sector, (III) hairdressing and beauty sector, (IV) food and catering sector, (V) cleaning sector, (VI) other sector with skin exposure to irritants and (VII) other sector without skin exposure to irritants or unemployed/pensioner.

Controls were apprentices in training for one of the following risk-proessions for CICD: (I) (geriatric) nurses, (II) metalworkers and mechanics, (III) hairdressers or (IV) cooks. They were recruited from the second and third school years at vocational schools in Osnabrueck, irrespective of skin abnormalities of the hands. The number of controls selected from each vocational training category was roughly based on the number of patients per job category. In total, 217 controls were included in the study. All controls completed a questionnaire on present and past skin abnormalities at the hands that are signs of dermatitis, on present and past flexural eczema and on wet work.

Patients and controls with chronic inflammatory diseases (e.g. rheumatoid arthritis, Crohn’s disease, systemic lupus erythematosus and psoriasis) were excluded. The inclusion criteria for patients and controls were: Caucasian and age \( \geq 18 \) years. Written informed consent was obtained from each subject before entering the study. The study was approved by the Ethical Committee of the University of Osnabrueck.
DNA sampling and isolation

For collection of DNA material, buccal mucosa cells were used obtained by rubbing the inside of the cheeks with a cotton swab on a plastic stick (Medispo, Oud Beijerland, the Netherlands). After the mouth was rinsed with water, two swabs were obtained. After sampling, each swab was placed into a 15 ml tube (Greiner Bio-one, Alphen a/d Rijn, the Netherlands) with 2 ml of lysis buffer (Puregene® Cell Lysis Solution, Gentra Systems, Minneapolis, MN, USA) to disrupt the cells and stabilize the DNA. Genomic DNA was extracted using a commercial DNA isolation kit (Puregene®, Gentra Systems, Minneapolis, MN, USA) based on a standard proteinase K digestion method according to the manufacturer’s protocol and described elsewhere.36 The tubes were stored at 4 °C up to 3 months before DNA isolation. The amount of DNA was quantified by absorbance at 260 nm, and an aliquot was diluted to a working concentration of 2-10 ng/μl.

FLG genotyping

The polymorphisms R501X and 2282del4 were genotyped by methods based on Palmer et al.13 Genotyping for R501X was performed by means of a fluorogenic 5’ nuclease PCR TaqMan® assay (Applied Biosystems, Foster City, CA, USA) using reagents obtained from Applied Biosystems (primers, probes and TaqMan® Universal PCR Master Mix without AmpErase® UNG) following the manufacturer’s protocol. R501X was typed by using the primer pair CCCTCTTGGGACGCTGAA and CACTGGAGGAAGACAAGGATCG, and the probes VIC-AGCTGTCTCATGCCT and 6FAM-AGCTGTCTCGTGCCT. Analyses were made on an ABI Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA, USA).

Genotyping for 2282del4 was performed by sizing a fluorescently labelled PCR fragment on a DNA sequencer (Abi Prism 3100 Genetic Analyzer, Applied Biosystems) using GeneMapper software (v4.0, Applied Biosystems). The polymorphism was typed by using the primer pair: TCCCGCCACCAGCTCCA and 6FAM-CTGATGGTGACCAGACACACAGCTGT. PCR reactions (20 μl) were performed using AmpliTaq Gold PCR Master Mix (Applied Biosystems). Reactions were amplified as follows: 95 °C (5 min), one cycle; 95 °C (15 s), 51 °C (15 s) and 72 °C (45 s), 33 cycles; and 72 °C (7 min), one cycle (MyCycler, Bio-Rad, Hercules, CA, USA). Fragments were diluted 1:40 and sized against ROX-350 size markers (Applied Biosystems) according to the manufacturer’s protocol. The wildtype allele was 190 bp, and the 2282del4 allele was 186 bp.
In both assays, DNA samples of known mutant-genotype (kindly provided by Prof. H. Traupe, Department of Dermatology, University of Münster, Germany) were used as a positive control. Further, each plate contained 2 non-template controls and 5 allelic controls. Random replicate samples (15% of all samples) were determined and showed intrasubject concordance rates of > 98%.

Statistics
The observed genotype frequencies were compared with the expected Hardy-Weinberg distribution by \( \chi^2 \) test. To estimate the risk of disease conferred by a particular genotype, we calculated the odds ratios (OR) with 95% confidence intervals (95%-CI) of being a patient using logistic regression comparing the heterozygous and homozygous variant allele genotype with the wildtype genotype. The \( \chi^2 \) test was used to compare the frequency of carriers of one or more FLG null alleles between the patients and controls or between subgroups of patients and controls. The \( \chi^2 \) test was also used to compare the frequencies of flexural eczema, type IV sensitizations, dry skin and skin abnormalities between carriers and non-carriers. The non-parametric Mann-Whitney U test was used to compare duration of skin exposure between carriers and non-carriers. P-values are two-sided. Patients or controls with incidental missing data were excluded from the analysis of that specific variable. The statistical analysis was performed using SPSS software version 12.0 (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of CICD patients and controls
Of the 206 CICD patients, the age was 40 ± 12 years (mean ± SD) and 64% were female. 93% of the patients reported that they were employed in a job with irritant exposure at the start of their hand eczema. The median age at the start of hand eczema was 33 years (range 0 – 60 years), the median duration of occupational irritant skin exposure until the onset of hand eczema was 6 years (range 0 – 41 years) and the median duration of hand eczema until the time of the investigation was 4 years (range 6 months – 50 years). The hand eczema was diagnosed as CICD without atopy in 27% of the patients, as CICD with atopic skin disposition in 24% and the remaining 49% was diagnosed as irritant-induced atopic hand eczema. The number of patients employed in each job sector at the start of their hand eczema is shown in Table 1. The
percentage of females was highest in the health care sector and lowest in the metal and construction sector (Table 1). The diagnosis CICD without atopy was most reported in the metal and construction sector and the diagnosis irritant-induced atopic hand eczema was most prevalent in the health care, the food and catering and the cleaning sectors.

Of the 217 controls, the age was $22 \pm 5$ years (mean $\pm$ SD); 59% were female. The control subjects were in vocational training to become a nurse (39%), a metalworker or a mechanic (24%), a hairdresser (28%), or a cook (9%). Forty percent of the controls reported to have skin abnormalities at the hands at present and 33% reported that skin abnormalities became first manifest after the start of their vocational training. The most reported present skin abnormalities of the hands were dry skin (76% of all controls with skin abnormalities), redness (47%), fissures (40%), itching (27%) and/or scaling (10%). The median duration since the start of the present skin abnormalities was 9 months and ranged from 1 week up to 36 years.

**Table 1. Number of patients, gender and sub-diagnosis of chronic irritant contact dermatitis (CICD) for the job categories at the start of hand eczema.**

<table>
<thead>
<tr>
<th>Sector</th>
<th>Frequency (%)</th>
<th>Females (%)</th>
<th>Sub-diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CICD without atopy (%)</td>
</tr>
<tr>
<td>Health care sector</td>
<td>67 (32.5)</td>
<td>95.5</td>
<td>14.9</td>
</tr>
<tr>
<td>Metal and construction sector</td>
<td>49 (23.8)</td>
<td>6.1</td>
<td>49.0</td>
</tr>
<tr>
<td>Hairdressing and beauty sector</td>
<td>26 (12.6)</td>
<td>84.6</td>
<td>23.0</td>
</tr>
<tr>
<td>Food and catering sector</td>
<td>12 (5.8)</td>
<td>75.0</td>
<td>16.7</td>
</tr>
<tr>
<td>Cleaning sector</td>
<td>12 (5.8)</td>
<td>83.3</td>
<td>8.4</td>
</tr>
<tr>
<td>Other, with skin exposure</td>
<td>26 (12.6)</td>
<td>38.5</td>
<td>38.4</td>
</tr>
<tr>
<td>Other, without skin exposure</td>
<td>14 (6.8)</td>
<td>92.9</td>
<td>14.3</td>
</tr>
<tr>
<td>Total</td>
<td>206 (100)</td>
<td>63.6</td>
<td>26.7</td>
</tr>
</tbody>
</table>

**FLG genotype in CICD patients and controls**

In both patient and control group, the genotype distributions of the R501X and 2282del4 polymorphisms did not deviate from the Hardy-Weinberg equilibrium. For both polymorphisms, we found no clear difference between the patient and control group in the frequency of carriers of one or more FLG null alleles (Table 2). In the patient group, 9.7% was carrier of
one or more *FLG* null alleles compared with 7.4% in controls (OR = 1.35, 95%-CI 0.68 – 2.69).

### Table 2. Genotype distribution of the R501X and 2282del4 FLG polymorphisms in chronic irritant contact dermatitis patients and controls.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>Patients n (%)</th>
<th>Controls n (%)</th>
<th>OR (95%-CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R501X</td>
<td>AA</td>
<td>198 (96.1)</td>
<td>213 (98.2)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Aa</td>
<td>8 (3.9)</td>
<td>4 (1.8)</td>
<td>2.15 (0.64-7.26)</td>
</tr>
<tr>
<td></td>
<td>aa</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>-</td>
</tr>
<tr>
<td>2282del4</td>
<td>AA</td>
<td>194 (94.2)</td>
<td>205 (94.5)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Aa</td>
<td>12 (5.8)</td>
<td>11 (5.1)</td>
<td>1.15 (0.50-2.67)</td>
</tr>
<tr>
<td></td>
<td>aa</td>
<td>0 (0)</td>
<td>1 (0.5)</td>
<td>-</td>
</tr>
<tr>
<td>Combined</td>
<td>AA</td>
<td>186 (90.3)</td>
<td>201 (92.6)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Aa*</td>
<td>20 (9.7)</td>
<td>15 (6.9)</td>
<td>1.44 (0.72-2.90)</td>
</tr>
<tr>
<td></td>
<td>aa**</td>
<td>0 (0)</td>
<td>1 (0.5)</td>
<td>-</td>
</tr>
</tbody>
</table>

AA, homozygous wildtype; Aa, heterozygous; aa, homozygous variant; *Heterozygous for R501X or 2282del4; **Homozygous for R501X or 2282del4; or compound heterozygous. OR, odds ratio; CI, confidence interval

### Findings in patient group

As in our study CICD patients with different sub-diagnoses were included, we investigated the association of *FLG* null alleles with these sub-diagnoses. In the subgroup of patients diagnosed as CICD without atopy, 3.6% (2/55) was carrier of a *FLG* null allele, 14.0% (7/50) was carrier in the subgroup diagnosed as CICD with atopic skin disposition, and 10.9% (11/101) of patients diagnosed as irritant-induced atopic hand eczema. The percentage of carriers in each of these three sub-diagnosis groups did not differ significantly from the control group (7.4% in the control group; 16/217, P > 0.15 for all comparisons). The percentage of carriers in patients diagnosed as ‘skin atopics’ i.e. CICD with atopic skin disposition or irritant-induced atopic hand eczema was 11.9% (18/151), which tended to be higher than the 3.6% (2/55) carriers in those diagnosed as CICD without atopy (P = 0.08), but not higher compared with the control group (P = 0.14).

We studied whether *FLG* null alleles might be a predisposing factor for present or past flexural eczema, which was recorded by the dermatologists in 35% of the patients. An in-
creased lifetime prevalence of flexural eczema was found for patients with a FLG null allele: 60% (12/20) of carriers of a FLG null allele had flexural eczema vs. 32% (55/170) of non-carriers (P = 0.014). Or by another approach, in patients with a history of flexural eczema 18% (12/67) was carrier of a FLG null allele and in those without flexural eczema 7% (8/123). The lifetime prevalence of flexural eczema was 33% (14/42) in patients diagnosed as CICD with atopic disposition and 55% (53/96) in patients diagnosed as irritant-induced atopic hand eczema.

Of all patients, 53% had one or more type IV sensitizations. The prevalence of one or more type IV sensitizations was 40% (8/20) in carriers of a FLG null allele, which did not differ significantly from the 55% (102/186) as reported in non-carriers (P = 0.20). A substantial part of the patients had a dry skin (60%), with a prevalence of 75% (15/20) in carriers and 58% (105/180) in non-carriers (P = 0.15).

We studied whether FLG null alleles shortens the duration of irritant skin exposure to the onset of hand eczema. The median duration of exposure before the start of hand eczema was 5.4 (range 0 - 29) years in carriers, which was not different from a median duration of 6.0 (range 0 - 41) years in non-carriers (P > 0.2).

Findings in control group

Of the controls, 40% reported skin abnormalities at the hands at the time of the investigation. We studied whether having one or more FLG null alleles might be a risk factor for skin abnormalities in this group. The prevalence of present skin abnormalities or past skin abnormalities which had started during their vocational training was 57% (8/14) in carriers of one or more FLG null alleles, compared with 41% (71/175) in non-carriers (P > 0.2). In carriers, the prevalence of having skin abnormalities at the hands before the start of the vocational training was 43% (6/14), which was significantly higher than 10% (17/175) in non-carriers (P < 0.001).

We studied whether FLG null alleles might be a predisposing factor for flexural eczema, which was reported in the questionnaires in 20% of the controls. The lifetime prevalence of self-reported flexural eczema was 36% (5/14) in carriers of one or more FLG null alleles, compared to a prevalence of 19% (35/184) in non-carriers (P = 0.13).
Discussion

This is the first study, to our knowledge, that investigated the association between polymorphisms in the FLG gene and occupational CICD. We found no effect (OR = 1.35, 95%-CI 0.68 - 2.69) of the FLG loss-of-function polymorphisms R501X and 2282del4 on risk of this skin disease.

As regards the control group, the frequency of carriers of R501X and 2282del4 null alleles in our apprentices (7.4%) was within the range of the reported frequency of carriers in German population controls (6-10%).

FLG null alleles are a predisposing factor for atopic dermatitis, therefore we expected to find an increased frequency of these alleles at least in the subgroup of patients with an atopic skin disposition (73% of all patients) or in patients with the sub-diagnosis irritant-induced atopic hand eczema (49% of all patients) compared with the control group. The frequency of 11.9% and 10.9% carriers, respectively, although not significant, was in line with this expectation. Among those patients diagnosed as CICD without atopy, we found a frequency of as low as 3.9% carriers, which may indicate that an impaired skin barrier without atopy (pathway B), as shown in Figure 1, is not very likely as a cause of CICD.

A large proportion (35%) of our patients had present or previous flexural eczema, which is a characteristic of atopic dermatitis. In this subgroup of patients, we found an increased frequency of FLG null allele carriers (18%), which is in agreement with the percentage of carriers of these risk alleles reported in the literature in adult patients with unspecified atopic dermatitis (range 16-21%). The lifetime prevalence of self-reported flexural eczema in carriers of a FLG null allele was 2 times higher compared with the wildtype genotype in the group of apprentices (36% vs. 19%), which is in agreement with a higher lifetime prevalence of flexural eczema (60%) in carriers compared with non-carriers (32%) in patients.

Recently published studies showed a lifetime prevalence of atopic dermatitis of 15-20% in children and adolescents in Western countries. As 20% of our apprentices reported a history of flexural eczema, which is a characteristic of atopic dermatitis, our data confirm the findings of Nyren et al. who reported that a subject with a history of atopic dermatitis in childhood does not seem to avoid a job with a high risk of hand eczema.

Recently, Lerbaek et al. published a population-based study, in which they investigated the association between FLG null alleles and self-reported hand eczema, including irritant and allergic contact dermatitis, atopic dermatitis, mixed forms and some subjects with vesicular
FLG loss-of-function polymorphisms and CICD

and hyperkeratotic hand eczema.\textsuperscript{44} Similar to our results, the FLG null alleles R501X and 2282del4 did not appear to be a risk factor for hand eczema.

The occurrence of signs of dermatitis at the hands during the vocational training of the apprentices was high, both in carriers (57\%) of a FLG null allele and in non-carriers (41\%). However, before the start of the vocational training, signs of dermatitis were four times more prevalent in carriers (43\%) than in non-carriers (10\%). An increase in prevalence of signs of hand dermatitis during vocational training was to be expected as the apprentices, in training for high risk occupations, perform wet work and/or are exposed to skin irritants. However, we do not have an explanation why the increase was less prominent among carriers of a FLG null allele compared with non-carriers. Future studies could investigate e.g. whether apprentices who are aware of their increased susceptibility take more protective measures.

Recently, we have reported that carriers of a FLG null allele have reduced amounts of natural moisturizing factors in their SC and reported dry skin.\textsuperscript{25} Therefore, we studied the relation between dry skin and FLG null alleles in patients. However, prevalence of dry skin seemed only slightly higher in carriers (75\%) compared with non-carriers (58\%). Possibly other factors present in our patient population might mask an effect of FLG deficiency. Filaggrin-deficient subjects were shown to have a reduced skin barrier function indicated by a higher transepidermal water loss,\textsuperscript{25} therefore one would expect an increased prevalence of allergic sensitizations. However, this factor was not different between carriers and non-carriers (40\% vs. 55\%), which is in line with the recently published findings of Lerbaek \textit{et al.} who did not find an association between contact allergy and FLG null alleles in a population-based study.\textsuperscript{44} More research is needed to determine whether the hypothesis on increased risk of allergic sensitizations in carriers of FLG null alleles can be disproved.

Recently, 11 other null-mutations in the FLG gene have been discovered in the European population,\textsuperscript{21,45} however, additional screening for these FLG polymorphisms will likely not change our results, as the sum of their frequencies is only \textasciitilde 2\% in the general population.\textsuperscript{21}

Our patient group consisted of cases of occupational CICD visiting the Dermatology Department in Osnabrueck. We will have missed some workers with a FLG null allele who reduced their exposure or moved to an occupation without irritant skin exposure after early appearance of hand eczema. This will have shifted our results towards the null-hypothesis. Our patients reported a median age at onset of hand eczema of 33 years, which was more than 10 years later than the median age of onset of 20 years reported in a large cohort of hairdressers\textsuperscript{46} or 21 to 25 years in the general population.\textsuperscript{46,47} This may indicate that we have missed several
patients who developed signs of hand eczema at a young age. The failure to show more clear
effects may also be due to the presence of patients with other causes than a high genetic sus-
ceptibility to CICD. The CICD in part of the patients might have been caused predominantly
by a high skin exposure to irritants. These patients reduce the impact of the susceptibility
genotypes under study in the patient group and consequently shift the ORs towards unity.
Such an effect will be aggravated if genetic susceptibility can be caused by several other
polymorphisms, e.g. functional polymorphisms in genes coding for inflammatory mediators
or proteins essential for the skin barrier. In view of the inevitable loss of genetically suscepti-
ble workers during their career preceding a case-control study, the contribution of a filaggrin
deficiency to CICD should preferably be determined by means of a prospective cohort study.
In such a study, the participants should be followed from the start of their vocational training.
An additional important advantage of a cohort study design is that the contributing effect of
the level of exposure can be studied. For the moment, our study suggests that FLG polymor-
phisms are not a substantial risk factor of CICD.
Acknowledgment

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