Filaggrin null mutations and association with contact allergy and allergic contact dermatitis: results from a tertiary dermatology clinic

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Background: Filaggrin null (FLG) mutations lead to skin barrier disruption with a reduced resistance towards exogenous agents and also influence the course of disease in atopic dermatitis.

Objectives: To examine the association between FLG mutations and contact allergy, polysensitization, hand eczema at first appearance of disease, occurrence, and course of dermatitis.

Methods: A venous blood sample from 430 individuals was genotyped for FLG mutations R501X and 2282del4 with polymerase chain reaction followed by typing through hybridization to paramagnetic polystyrene beads and analysis on a BioPlex 200. All individuals had a minimum of one positive patch test reaction.

Results: In all, 3.5% were 2282del4 heterozygote and 5.1% were R501X heterozygote. An odds ratio (OR) of 1.49 [95% confidence interval (CI) 0.74–3.00] was found for nickel allergy, OR 0.84 (95% CI 0.41–1.74) for polysensitization, OR 0.78 (95% CI 0.25–2.43) for dermatitis, OR 0.96 (95% CI 0.48–1.92) for hand eczema at debut, OR 1.25 (95% CI 0.99–1.57) for duration of disease, and OR 0.76 (95% CI 0.59–0.97) for age at onset.

Conclusions: No association between nickel allergy, polysensitization, hand eczema at first appearance or occurrence of dermatitis, and FLG mutations was found. However, patients with FLG mutations had an earlier age of onset compared with the wild-type genotype and a trend towards longer duration of disease.

Key words: allergic contact dermatitis; contact allergy; filaggrin null mutations; hand eczema; polysensitization.

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risk of allergic type I sensitization (aeroallergens), allergic rhinitis, and asthma in combination with atopic dermatitis (6). The mechanism behind is unknown, but it is proposed that the allergic diseases develop because of penetration of protein allergens through the skin. Increased penetration of protein allergens through a filaggrin-deficient skin and a secondary local and systemic inflammatory response has been verified in animal models (3, 4). Theoretically, a suboptimal skin barrier may also increase the risk of sensitization to contact allergens and probably the number of contact allergies acquired because of increased penetration. One recent study in filaggrin-deficient mice reported a reduced threshold for development of hapten-induced allergic contact dermatitis and an increased propensity to irritant contact dermatitis (5). Because the filaggrin deficiency is permanent, the course of dermatitis may also be affected or dermatitis may develop at an earlier age because of lack of resistance to exogenous agents.

This study investigated the association between filaggrin null mutations R501X and 2282del4 and contact allergy including nickel allergy, polysensitization, hand eczema at first appearance (debut) of disease, and occurrence and course of dermatitis measured as duration of disease and age of onset of symptoms, respectively, in a tertiary dermatology clinic.

**Methods**

**Study population**

The study population consisted of 430 Caucasian individuals who were patch tested with the European baseline series (23 allergens) and had a minimum of one positive patch test reaction. In all, 77.7% were women. The average age at the time of patch testing was 48.0 years (SD ± 13.6). The study population was recruited from a questionnaire survey population (7, 8). The study was approved by the ethics committee of the capital region of Copenhagen. Written informed consent was obtained from all participants in accordance with the principles of the Helsinki Declaration.

**Patch testing**

Patch testing was performed with Finn chambers® (Epitest Ltd Oy, Tuusula, Finland), Scanpor® tape (Norgesplaster A/S, Alpharma, Vennesla, Norway), and TROLAB® (Hermal, Reinbek, Germany) patch test allergens applied to the upper back. The occlusion time was 48 hr, and readings were performed on D2, D3, or D4, and D7 according to the recommendation from the International Contact Dermatitis Research Group (9). A 1+, 2+, and 3+ reaction was interpreted as a positive reaction.

**Phenotype definitions**

**Polysensitization** was defined as three or more contact allergies according to the recent reviews (10, 11). The duration of disease, age at onset of dermatitis, localization of dermatitis at the time of debut, diagnosis of dermatitis, and diagnosis of atopic dermatitis were based on data from the questionnaire survey (7, 8). The validated UK Working Party’s Diagnostic Criteria, question-only version, identified individuals with atopic dermatitis (12). A **diagnosis of dermatitis** was defined as an affirmative answer to the question: ‘Have you ever had dermatitis?’ The **duration of disease** was measured in years by subtracting the debut year from the year where the last dermatitis episode occurred. The duration of disease measures the total duration between first and last dermatitis episode regardless of intermittent dermatitis-free periods. The year of onset of dermatitis was determined by asking participants: ‘What year did the dermatitis first appear?’. **Age at onset** was measured in years by subtracting the birth year from the year of debut of dermatitis. The occurrence of **hand eczema** was determined by asking where the dermatitis was located at the time of debut.

**Genotyping**

Venous blood samples were collected from each participant and stored at −20°C. Each participant was genotyped for the R501X and 2282del4 null mutations. The genotyping assay was based on polymerase chain reaction followed by typing through hybridization to mutation and wild-type-specific paramagnetic polystyrene beads (Luminex, Austin, TX, USA) and analysed on a BioPlex 200® (Biorad, Hercules, CA, USA).

**Statistics**

Hardy–Weinberg equilibrium was assessed using the web version of Genepop (http://genepop.curtin.edu.au/), and the genotype variants were in equilibrium ($P > 0.05$).

Associations between filaggrin null mutations and the different phenotypes examined were performed with logistic regression analyses. One logistic regression model for each phenotype was performed. The models were appropriately adjusted for atopic dermatitis, sex, age, and polysensitization. The effect of filaggrin null mutations on prevalence of hand eczema may vary in accordance with occurrence or lack of atopic dermatitis (13). Therefore, a second logistic regression analysis was performed for hand eczema where an interaction term between atopic dermatitis and filaggrin mutations was included as an independent variable. A second
logistic regression analysis was also performed when examining the association between nickel contact allergy and filaggrin null mutations. In this second analysis, ‘nickel allergy and occurrence of dermatitis’ was used as dependent outcome instead of nickel allergy alone to increase the level of relevant reactions.

The study population was divided into four groups based on atopic dermatitis and mutation status. Group 1 consisted of individuals without atopic dermatitis and without filaggrin mutations. Group 2 consisted of individuals without atopic dermatitis but with filaggrin mutations; group 3 with atopic dermatitis but without filaggrin mutations; and group 4 with both atopic dermatitis and filaggrin mutations. The distribution of the different phenotypes across these four groups was compared with trend test for nominal data and Kruskal–Wallis test for continuous data. Normal distribution of continuous data was tested with Kolmogorov–Smirnov tests and was not normally distributed for all the four groups.

Throughout the analyses, a $P$ value of 0.05 was regarded as significant. All statistical analyses were performed in the statistical software programme SPSS® version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

All 430 individuals were successfully genotyped. A total of 37 (8.6%) individuals were filaggrin mutation carriers; 15 (3.5%) were 2282del4 heterozygote and 22 (5.1%) were R501X heterozygote. No homozygote or compound heterozygote carriers were detected.

Table 1 shows phenotype status for, respectively, null mutation carriers and wild-type individuals, and Table 2 shows phenotype status for null mutation carriers and wild-type individuals stratified by atopic dermatitis. In all, 35.1% and 38.2% of, respectively, filaggrin null mutation carriers and wild-type individuals had three or more contact allergies (= polysensitization) [odds ratio (OR) 0.84, 95% confidence interval (CI) 0.41–1.74]. Also, filaggrin null mutations were not associated with occurrence of dermatitis or hand eczema (Table 1). Occurrence of hand eczema was determined at the time of debut of dermatitis. The results for hand eczema did not

### Table 1. Association between different phenotypes and filaggrin null mutation carrier status

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Wild-type</th>
<th>R501X/2282del4 combined</th>
<th>Logistic regression analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (No.) or median years (IQR)</td>
<td>% (No.) or median years (IQR)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>All</td>
<td>91.4% (393)</td>
<td>8.6% (37)</td>
<td>–</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>91.3% (356/390)</td>
<td>89.2% (33/37)</td>
<td>0.78 (0.25–2.43)*</td>
</tr>
<tr>
<td>Hand eczema</td>
<td>58.6% (228/389)</td>
<td>58.3% (21/36)</td>
<td>0.96 (0.48–1.92)†</td>
</tr>
<tr>
<td>Polysensitization</td>
<td>38.2% (150/393)</td>
<td>35.1% (13/37)</td>
<td>0.84 (0.41–1.74)‡</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>24.5 years (IQR 13–39)</td>
<td>35.0 years (IQR 15.25–48)</td>
<td>1.25 (0.99–1.57)*</td>
</tr>
<tr>
<td>Age at onset</td>
<td>26 years (IQR 14–42)</td>
<td>16 years (IQR 7–30.25)</td>
<td>0.76 (0.59–0.97)§</td>
</tr>
</tbody>
</table>

IQR, interquartile range; OR, odds ratio; CI, confidence interval.

*R Adjusted for atopic dermatitis and polysensitization.

†Adjusted for atopic dermatitis, sex, age, and polysensitization.

‡Adjusted for atopic dermatitis, sex, and age.

§Adjusted for atopic dermatitis.

### Table 2. Phenotypes stratified by four combinations of atopic dermatitis and filaggrin mutation status

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Group 1 (- atopic dermatitis, - filaggrin mutation)</th>
<th>Group 2 (- atopic dermatitis, + filaggrin mutation)</th>
<th>Group 3 (+ atopic dermatitis, - filaggrin mutation)</th>
<th>Group 4 (+ atopic dermatitis, + filaggrin mutation)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>58.6% (252/430)</td>
<td>4.9% (21/430)</td>
<td>32.8% (141/430)</td>
<td>3.7% (16/430)</td>
<td>–</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>87.6% (218/249)</td>
<td>81.0% (17/21)</td>
<td>97.9% (138/141)</td>
<td>100% (16/16)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Hand eczema</td>
<td>56.0% (139/248)</td>
<td>57.1% (12/21)</td>
<td>63.1% (89/141)</td>
<td>60.0% (9/15)</td>
<td>0.21*</td>
</tr>
<tr>
<td>Polysensitization</td>
<td>31.7% (80/252)</td>
<td>14.3% (3/21)</td>
<td>49.6% (70/141)</td>
<td>62.5% (10/16)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Duration of disease (median years, IQR)</td>
<td>19 (10–37)</td>
<td>27 (15.25–50.5)</td>
<td>31 (17–43.75)</td>
<td>36.5 (16.75–47)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Age at onset (median years, IQR)</td>
<td>28 (18–44)</td>
<td>18.5 (11.25–36.25)</td>
<td>20 (6.75–36.25)</td>
<td>11.5 (2–29.5)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Nickel allergy</td>
<td>30.9% (77/249)</td>
<td>47.6% (10/21)</td>
<td>39.6% (55/139)</td>
<td>43.8% (7/16)</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

IQR, interquartile range.

* Trend test.

†Kruskal–Wallis test.
change when an interaction between atopic dermatitis and filaggrin mutations was included in the logistic regression analysis (interaction term, \( P = 0.72 \)).

A large difference in duration of disease was obvious between the wild-type and the mutation genotypes (Table 1), and the proportion of null mutation carriers increased with increasing duration of disease (Fig. 1). The association between duration of disease and filaggrin mutation status was borderline significant (\( P = 0.056 \)).

Individuals with null mutations were on average 10 years younger at the time of onset of dermatitis than individuals without mutations (Table 1) with the largest proportion of mutation carriers debuting between 0 and 9 years of age (Fig. 2).

Nickel-allergic individuals were over represented among filaggrin mutation carriers (45.9%) compared with individuals without filaggrin mutations (34.0%); however, it was not significant [OR 1.49 (95% CI 0.74–3.00)] (Table 3). It did not change the results when a modified variable ‘nickel allergy and dermatitis’ was used as dependent outcome instead of nickel allergy alone (\( P = 0.26 \)). None of the other 22 allergens tested in the European baseline series was associated with filaggrin null mutations (Table 3).

**Discussion**

The combined filaggrin mutation carrier frequency reached 8.6% and was similar to the frequency in a Danish general population (14). Similar general population studies from Germany and United Kingdom also report allele frequencies around
Table 3. Association between filaggrin null mutation carrier status and contact allergy to European baseline allergens

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Wild-type, % (No)</th>
<th>R501X/2282del4 combined, % (No)</th>
<th>Logistic regression analyses OR (CI 95%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium dichromate allergy</td>
<td>14.6 (57/391)</td>
<td>10.8 (4/37)</td>
<td>0.66 (0.22–1.95)</td>
<td>0.45</td>
</tr>
<tr>
<td>Neomycin allergy</td>
<td>7.4 (29/391)</td>
<td>2.7 (1/37)</td>
<td>0.37 (0.05–2.84)</td>
<td>0.34</td>
</tr>
<tr>
<td>Thiuram mix allergy</td>
<td>12.7 (50/393)</td>
<td>5.4 (2/37)</td>
<td>0.39 (0.09–1.70)</td>
<td>0.21</td>
</tr>
<tr>
<td>Para-phenylenediamine allergy</td>
<td>7.1 (28/392)</td>
<td>8.1 (3/37)</td>
<td>1.17 (0.34–4.06)</td>
<td>0.81</td>
</tr>
<tr>
<td>Cobalt allergy</td>
<td>13.3 (52/391)</td>
<td>13.5 (5/37)</td>
<td>0.98 (0.36–2.64)</td>
<td>0.96</td>
</tr>
<tr>
<td>Benzocaine allergy</td>
<td>1.3 (5/393)</td>
<td>0 (0/37)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Formaldehyde allergy</td>
<td>13.0 (51/392)</td>
<td>11.1 (4/36)</td>
<td>0.81 (0.27–2.40)</td>
<td>0.70</td>
</tr>
<tr>
<td>Colophonium allergy</td>
<td>15.3 (60/393)</td>
<td>16.2 (6/37)</td>
<td>1.06 (0.42–2.66)</td>
<td>0.91</td>
</tr>
<tr>
<td>Clioquinol allergy</td>
<td>2.5 (10/393)</td>
<td>2.7 (1/37)</td>
<td>1.45 (0.17–12.2)</td>
<td>0.73</td>
</tr>
<tr>
<td>Myroxylon pereirae allergy</td>
<td>20.9 (82/392)</td>
<td>21.6 (8/37)</td>
<td>1.08 (0.47–2.47)</td>
<td>0.86</td>
</tr>
<tr>
<td>IPPD allergy</td>
<td>1.8 (7/393)</td>
<td>0 (0/37)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lanolin allergy</td>
<td>2.3 (9/393)</td>
<td>2.7 (1/37)</td>
<td>1.10 (0.13–8.99)</td>
<td>0.93</td>
</tr>
<tr>
<td>Mercapto mix allergy</td>
<td>4.8 (19/393)</td>
<td>2.7 (1/37)</td>
<td>0.72 (0.09–5.70)</td>
<td>0.76</td>
</tr>
<tr>
<td>Epoxy resin allergy</td>
<td>3.1 (12/393)</td>
<td>2.7 (1/37)</td>
<td>0.83 (0.10–6.64)</td>
<td>0.86</td>
</tr>
<tr>
<td>Paraben mix allergy</td>
<td>1.5 (6/393)</td>
<td>2.7 (1/37)</td>
<td>1.96 (0.22–17.7)</td>
<td>0.55</td>
</tr>
<tr>
<td>PTBFR allergy</td>
<td>6.4 (25/393)</td>
<td>2.7 (1/37)</td>
<td>0.44 (0.06–3.36)</td>
<td>0.43</td>
</tr>
<tr>
<td>Fragrance mix allergy</td>
<td>35.2 (138/392)</td>
<td>32.4 (12/37)</td>
<td>0.90 (0.44–1.86)</td>
<td>0.77</td>
</tr>
<tr>
<td>Quaternium-15 allergy</td>
<td>5.1 (20/393)</td>
<td>11.1 (4/36)</td>
<td>2.43 (0.76–7.78)</td>
<td>0.14</td>
</tr>
<tr>
<td>Nickel allergy</td>
<td>34.0 (132/388)</td>
<td>45.9 (17/37)</td>
<td>1.49 (0.74–3.00)</td>
<td>0.27</td>
</tr>
<tr>
<td>MCI/CI allery</td>
<td>9.2 (36/391)</td>
<td>5.4 (2/37)</td>
<td>0.55 (0.13–2.38)</td>
<td>0.42</td>
</tr>
<tr>
<td>Mercaptobenzothiazole allergy</td>
<td>5.1 (20/391)</td>
<td>2.8 (1/36)</td>
<td>0.68 (0.09–5.37)</td>
<td>0.71</td>
</tr>
<tr>
<td>Primin allergy</td>
<td>2.8 (11/392)</td>
<td>2.7 (1/37)</td>
<td>0.89 (0.11–7.27)</td>
<td>0.91</td>
</tr>
<tr>
<td>Sesquiterpene lactone mix allergy</td>
<td>6.7 (24/360)</td>
<td>5.9 (2/34)</td>
<td>0.88 (0.19–4.07)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

IPPD, N-isopropyl-N-phenyl-p-phenylenediamine; PTBFR, p-tertiary butylphenol formaldehyde resin; MCI/CI, methylchloroisothiazoline (and) methylisothiazoline.

*Adjusted for atopic dermatitis, age, and sex.

9% (15, 16). Higher allele frequencies (18–27%) have been found in case–control and family studies where study subjects were included based on diagnosis of atopic dermatitis (17), but even in one recent intervention study where children were included based on an atopic dermatitis diagnosis, the filaggrin allele frequency only reached 8.3% (18).

It has been hypothesized that filaggrin mutations may be associated with nickel contact allergy (19). Nickel is accumulated on the top layers of the epidermis, and histidine-rich polypeptides are nickel-chelating agents (20, 21). Filaggrin is histidine rich and found in the upper layers of the epidermis (22), and the level of histidine is reduced in filaggrin mutation carriers (23). Nickel may therefore penetrate the epidermis more rapidly when filaggrin is lacking. We found a positive association between filaggrin null mutations R501X and 2282del4 combined and nickel contact allergy; however, it was not significant. One other study also found an association between filaggrin null mutations and nickel contact allergy but only when a positive patch test to nickel sulfate appeared in the context of an anamnesis of skin reactions to jewellery (24), and another study only found an association between nickel contact allergy and filaggrin mutations when an adjusted analysis on women who were not ear pierced were performed (14).

Individuals with three or more contact allergies (polysensitization) are generally regarded as a particular susceptible group among contact allergic individuals (10, 11). They show functional changes in induction and elicitation studies (25, 26), occur more frequently than expected by chance (25, 27), and develop more new positive patch test reactions compared with patients with one allergy when tested additional times (28). Polymorphism in the interleukin-16 gene and tumour necrosis factor-α gene has been associated with polysensitization (29, 30). But has also been associated with contact allergy in general, irritant contact dermatitis, and atopic dermatitis (29–32). A suboptimal skin barrier caused by lack of filaggrin may influence the number of contact allergies; however, no association between polysensitization and filaggrin null mutations was found in this study.

Contact allergens are less than 500 Da (33). Protein allergens are much larger. The increased penetration of allergen through a filaggrin-deficient skin may not concern contact allergens. Contact allergens may penetrate the epidermis regardless of the filaggrin status because of the small size, whereas protein allergens only penetrate in the case of a defect barrier. This hypothesis can explain the lack of association between filaggrin null mutations and contact allergy in general and polysensitization but needs further investigation.
Environmental exposure is an absolute requirement for contact sensitization to develop, but it is not the sole driver. It was not possible to quantify the environmental exposure in the individuals in the present study population and accordingly no adjustments could be performed. This may well explain the lack of significant association between filaggrin null mutations and contact allergy/polysensitization as illustrated in one recent study where a strong environmental risk factor for nickel contact allergy, ear piercing, was accounted for. No association between filaggrin null mutations and nickel contact allergy was apparent in ear-pierced women; however, a significant association was showed when examined in women who never had been ear pierced (14). Unfortunately, we did not have data on ear-piercing status and accordingly no adjustment for this parameter could be performed in this study.

Atopic dermatitis is a strong risk factor for hand eczema, but heritability of hand eczema is not exclusively explained by comorbidity of atopic dermatitis (34). In 2007, Lerbaek et al. (35) reported a filaggrin mutation carrier frequency of 13% for hand eczema patients and 7–8% for controls. The differences were not statistically significant; however, the study lacked statistical power. Most recently, Molin et al. (36) could not detect any association between chronic hand eczema and filaggrin null mutations, but a significant association was found between individuals with concomitant hand eczema and excessive daily exposure to water or irritants and filaggrin null mutations. A trend toward an association between filaggrin null mutations and chronic hand eczema caused by a combination of allergic and irritant contact dermatitis was also observed but was not statistically significant. We were not able to show any association between hand eczema and filaggrin null mutations.

The effect of filaggrin null mutations on prevalence of hand eczema may only concern distinct subtypes of hand eczemas. This was recently shown where the effect of filaggrin null mutations only increased the risk of hand eczema in subjects with atopic dermatitis but not in subjects without atopic dermatitis (13), and modest evidence of an association between filaggrin mutations and irritant hand dermatitis has also been reported (36, 37). It was not possible to estimate the irritant exposure and the amount of wet work or quantify other environmental influences in the individuals in the present study population and accordingly no adjustment for such parameters could be performed. The environmental influence may be so great that it out weights any genetic influence if not accounted for. Furthermore, despite attempts to account for the variable effect of filaggrin null mutations in, respectively, individuals with and without atopic dermatitis, we were not able to show any association between filaggrin mutations and hand eczema. In this study, the occurrence of hand eczema was estimated at the time of debut of dermatitis, whereas hand eczema typically develops at a later stage of atopic dermatitis.

Even though patients with filaggrin null mutations were not more likely to have dermatitis, the ones with dermatitis were on average younger at the time of debut of dermatitis than individuals with the wild-type genotype. A trend towards a longer duration of disease was also observed. It was marginally significant. Reduced levels of filaggrin in the skin because of a heterozygote filaggrin mutation status seem to influence the course of disease in line with the reported strong associations between filaggrin null mutations and early debut, severity, and persistency of disease in atopic dermatitis (6).

Neither nickel contact allergy, polysensitization, dermatitis, nor hand eczema at the time of debut of symptoms was associated with filaggrin null mutations in the present study; however, the filaggrin mutations did seem to influence the course of dermatitis. Subtle associations require large datasets to be detected. We cannot exclude that those associations that did not reach statistical significance in the present study would have done so in larger datasets. Furthermore, contact dermatitis, contact allergies, and hand eczema are complex traits influenced mainly by environmental exposures but also to some degree by genetics and perhaps also influenced by gene–environment interactions. This contributes to the difficulty of determining the genetic contribution. Some recent studies underline the importance of accounting for environmental influences and subclassifications. However, our findings suggest that the filaggrin null mutation genotype influences the course of dermatitis.

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