

No remarkable differences in rates of sensitization to common type I and IV allergens between *FLG* loss-of-function mutation carriers and wild-type subjects

Lilla Landeck¹, Maaïke Visser², Christoph Skudlik¹, Richard Brans¹, Sanja Kezic* and Swen Malte John*

¹Department of Dermatology, Environmental Medicine and Health Theory, University of Osnabrück, 49090 Osnabrück, Germany and ²Coronel Institute of Occupational Health, Academic Medical Centre, University of Amsterdam, 1100 DE Amsterdam, The Netherlands

doi:10.1111/cod.12109

Summary

Background. Loss-of-function mutations in the filaggrin gene (*FLG*) have been associated with reduced skin barrier function, possibly allowing increased penetration of irritants and allergens.

Objectives. To study whether *FLG* loss-of-function mutation carriers show different rates of sensitization to common type I and IV allergens among patients referred for occupational contact dermatitis of the hands.

Materials and Methods. Four hundred and ninety-six Caucasian patients were genotyped for four *FLG* null mutations and patch tested with the European baseline series. In addition, 431 patients underwent prick testing with common type I allergens.

Results. Overall, 67 patients showed a heterozygous mutation in the *FLG* alleles R501X, R2447X, S3247X, and/or 2282del4. Sensitization rates for type I allergens from a European prick test series did not show significant differences between *FLG* loss-of-function mutation carriers and wild-type subjects. For type IV allergens, significantly more *FLG* loss-of-function carriers were found to be sensitized to lanolin and *p*-tert-butylphenol-formaldehyde resin.

Conclusions. Probably a variety of immunological mechanisms other than that resulting from the filaggrin system have an impact on allergic sensitization to a greater degree. Larger cohorts may be necessary to increase the statistical power of the findings presented regarding type I and IV sensitization.

Key words: allergens; contact dermatitis; filaggrin gene; loss-of-function mutations; patch test; prick test.

Loss-of-function mutations in the filaggrin gene (*FLG*) have been linked to disrupted skin barrier function and a strong predisposition to the development of atopic eczema. To date, 49 *FLG* mutations have been reported, and five are highly prevalent within European

populations (1). Among Caucasians, an estimated 10% are carriers of at least one loss-of-function mutation (1). Filaggrin aggregates keratin filaments in the stratum corneum, and is responsible for the mechanical strength of the skin. Moreover, it forms an important permeability barrier to water, microbes, and allergens, and provides defence by maintaining skin integrity.

Considering the key role of filaggrin in constituting and maintaining an effective skin barrier against the environment, it can be hypothesized that a constitutionally altered skin barrier in *FLG* loss-of-function mutation carriers will facilitate the penetration of skin irritants and

Correspondence: Lilla Landeck, Sedanstr. 115, D3, 49090 Osnabrück. Tel: +49-541-4051810; Fax: +49-541-9692445. E-mail: llandeck@uos.de

*These authors contributed equally to this manuscript.
Conflicts of interest: The authors have no conflicts of interest to declare.

Accepted for publication 6 May 2013

allergens, leaving them more susceptible to irritant and allergic contact dermatitis.

Recently, *FLG* loss-of-function mutations have been associated with irritant contact dermatitis (2, 3). A case–control study of our group showed that 15.9% of patients with irritant contact dermatitis were heterozygous carriers for the combined null alleles R501X, 2282del4, R2447X, and S3247X, in contrast to 8.3% of the controls, translating to a doubled risk of acquiring irritant contact dermatitis (2). Even after correction for the effect of atopic eczema (AE), the risk for *FLG* loss-of-function mutation carriers was significantly increased [odds ratio (OR) 1.62, 95% confidence interval (CI) 1.01–2.58] (2).

As additional sensitization to type I or IV allergens may worsen the course of irritant contact dermatitis and AE, there is a need to understand the possible role of *FLG* loss-of-function mutations in allergic disorders. In murine models, it has been shown that *FLG* deficiency alone is able to provoke a paracellular barrier abnormality, thereby reducing inflammatory thresholds for topical haptens and irritants (4). Another study using a mouse model has shown that topical allergen application to mice homozygous for murine *FLG* results in inflammatory infiltrates and enhanced cutaneous allergen priming, with the subsequent development of allergen-specific antibody responses (5).

The objective of this investigation was to study whether patients with *FLG* loss-of-function mutations show aberrant rates of sensitization to common type I and IV allergens from the European prick test series and baseline patch test series as compared with wild-type carriers among patients with occupational contact dermatitis of the hands.

Methods

This study was approved by the ethics committee of the University of Osnabrück, and was conducted in accordance with the Declaration of Helsinki.

Study population

After written informed consent for study participation had been obtained, 527 consecutive Caucasian patients, aged 18–65 years, referred with a primary diagnosis of long-standing occupational contact dermatitis of the hands, were included. Patients with a history of other chronic inflammatory disorders (e.g. rheumatoid arthritis and psoriasis) or a primary diagnosis of AE were excluded. The diagnosis of contact eczema was based on patient history, exposure to irritants/allergens, clinical distribution, the

presence of skin lesions, and the exclusion of other dermatological entities. For each patient, an experienced dermatologist performed a full examination of the skin, and a detailed medical history was recorded with a standardized questionnaire.

Atopic skin diatheses, allergic rhinoconjunctivitis, and asthma

The presence of additional atopic skin diatheses was diagnosed according to ongoing or past flexural eczema and/or if at least 10 points on the Erlangen atopy score were reached (6).

Subjects were classified as having asthma or rhinoconjunctivitis when they reported a physician's diagnosis of it and showed typical clinical signs (asthma – the presence of at least one wheezing period; allergic rhinoconjunctivitis – itching, sneezing, rhinorrhoea, congestion of the nose, and non-nasal symptoms, including tearing, eye-itching and redness within the last 12 months). Allergic type I sensitization was defined as the presence of at least one positive prick test result. Total IgE antibodies were measured with a fluorescent enzyme immunoassay (CAP-FEIA; Pharmacia, Uppsala, Sweden). A raised total IgE was taken to be > 100 kU/l.

Filaggrin genotyping

Subjects were genotyped for the four most prevalent *FLG* mutations – 2282del4, R501X, R2447X, and S3247X – in the European population, as described previously (7). Genotyping was conducted on saliva obtained from buccal swabs.

Skin testing

Subjects were tested only during periods of quiescent skin inflammatory disease, with test sites free of inflammation, and if systemic antihistamines and topical anti-inflammatory treatments on the test sites were stopped for at least 1 week. Further immunomodulating systemic medication (e.g. corticosteroids) and/or ultraviolet treatment had to be stopped for 4 weeks prior to testing.

Prick testing

When type I-mediated diseases were expected, subjects underwent prick testing. Subjects were tested with 23 allergen extracts, histamine, and a negative control solution according to standard guidelines (8). Prick tests were performed on the flexor side of the forearm with standardized test extracts. Allergens were purchased from ALK-Abelló (Wedel, Germany). In addition, subjects

were tested with latex in three different dilutions: 1:100, 1:10, and 1:1. Test reactions were read after 20 min (8). Results were regarded as positive if the mean wheal diameter was ≥ 3 mm at prick testing. For statistical analysis, results were allocated to the following groups: mite, grass pollen, tree pollen, dog dander, cat dander, mould, ribwort/mugwort/parietaria, and others. Others were excluded from further analyses, and are not shown here.

Patch testing

Subjects underwent patch testing with the European baseline series, and, depending on the individual's history and clinical picture, with further supplemental series (preservatives, cosmetic series, etc.). In this analysis, only the European baseline series was included. Test procedures and readings were conducted with a standardized technique in accordance with international recommendations by applying Haye's test chambers (HAL, Düsseldorf, Germany) affixed with Fixomull® stretch tape (Beiersdorf, Hamburg, Germany) to the upper back (9). Allergens were purchased from Almirall-Hermal (Reinbek, Germany). Tests remained in place for 1 day, and sites were initially evaluated at D1, and again at D2, D3, and D4, and, in some cases, at D7. Only D3 readings are included in this report. Patch test readings were classified according to the International Contact Dermatitis Research Group recommendations as allergic (+, ++, +++), questionable, irritant, or negative (9). The relevance of positive results was determined by assessment of past history of contact with the allergen and whether expressed symptoms were consistent with the clinical picture and history.

Data analyses and statistics

The observed genotype frequencies were compared with the expected Hardy–Weinberg distribution by use of the chi-square test (<http://www.oege.org/software/hwemr-calc.shtml>). For each type I and type IV allergen, sensitization rates were compared between *FLG* wild-type subjects and *FLG* loss-of-function mutation carriers by use of the chi-square test. For allergens showing a significant effect of *FLG* loss-of-function mutations on 'sensitization rate', 'sensitization to at least one allergen', and 'polysensitization' to type I and type IV allergens, the combined effects of *FLG* loss-of-function mutations and atopic skin diathesis were studied in a multiple logistic regression model with backward selection of variables. *FLG* loss-of-function mutations, atopic skin diathesis and the interaction term *FLG* loss-of-function mutations $\times$ atopic skin diathesis were entered

as covariates. The statistical analyses were performed with IBM SPSS™ version 19.0 (SPSS Inc., Chicago, IL, USA). Polysensitization was defined as sensitization to at least three chemically unrelated allergens (10).

Results

Our study included 496 subjects who were successfully genotyped and patch tested; 31 patients were excluded because of failure of *FLG* genotyping or patch testing. Four hundred and thirty-one subjects were also prick tested. Subjects were aged 18–65 years (mean 41 ± 12 years), and 301 (60.7%) were female. Three hundred and fifty-five (71.6%) of the total study population were diagnosed with additional atopic skin diathesis. The number of patients with underlying atopic skin diathesis was significantly greater among *FLG* loss-of-function mutation carriers (57/67, 85% $p = 0.01$, OR 2.5, 95% CI 1.24–5.05) than among wild-type subjects (298/429, 70%).

Of the study population, 67 subjects were carriers of a *FLG* loss-of-function mutation (13.5%). Among these, we identified 4 compound heterozygotes (carriage of two different *FLG* mutations) and 63 heterozygotes. No homozygous carriers were seen.

Regarding type I allergies, no significant differences between *FLG* loss-of-function mutation carriers and wild-type subjects were observed for common allergens, allergic asthma, or allergic rhinoconjunctivitis ($p > 0.05$). A higher polysensitization rate (sensitization to three or more type I allergens) was detected in *FLG* loss-of-function mutation carriers ($p = 0.06$), as shown in Table 1. However, after correction for the effect of atopic skin diathesis, the p -value for the adjusted OR of *FLG* was 0.125. In contrast, regression analysis including *FLG* and atopic skin diathesis as risk factors showed a significant impact of atopic skin diathesis on the frequencies of both type I sensitization and type I polysensitization (Table 2).

In 85 subjects, total IgE was measured (wild-type subjects, $n = 73$; *FLG* loss-of-function mutation carriers, $n = 12$). In both groups, the mean value of total IgE was increased; in *FLG* loss-of-function mutation carriers, the mean value of total IgE exceeded the standard level by four-fold. However, because of low statistical power, this finding did not reach the level of significance of $p \leq 0.05$.

Subgroup analysis to detect possible differences between compound heterozygous and heterozygous subjects did not show significantly different results (detailed results not shown).

Regarding type IV allergens, sensitization to lanolin and p -tert-butylphenol-formaldehyde resin (PTBP-FR) were significantly more often seen in *FLG* loss-of-function carriers (Table 3). Multiple regression analysis showed

Table 1. Rates of sensitization to common allergens in a European prick test series, rates of polysensitization, patients with allergic rhinitis and allergic asthma and total IgE among wild-type subjects and *FLG* loss-of-function mutation carriers

Allergens	<i>FLG</i> wild-type subjects (n = 371) Positive, n (%)	<i>FLG</i> loss-of-function mutation carriers (n = 60) Positive, n (%)
Mite	99 (26.7)	17 (28.3)
Grass pollen	97 (26.1)	19 (31.7)
Tree pollen	85 (22.9)	18 (30)
Dog dander	28 (7.5)	6 (10)
Cat dander	54 (14.6)	13 (21.7)
Mould	24 (6.5)	2 (3.3)
Natural rubber latex	18 (4.9)	2 (3.3)
Ribwort/mugwort/ parietaria	41 (11.1)	9 (15)
At least one type I sensitization	195 (52.6)	30 (50.0)
Polysensitization (sensitization to three or more allergens)	47 (12.7)	13 (21.7)
Median total IgE* in kU/l (range)	139 (1–3331)	401 (14–5000)
Allergic rhinitis	137 (36.9)	23 (38.3)
Allergic asthma	71 (19.1)	15 (25.0)

No statistically significant differences between the two groups were found.

*Measured in 85 patients (73 wild-type subjects; 12 *FLG* loss-of-function mutation carriers).

that this effect of *FLG* loss-of-function mutations on sensitization rate was independent of atopic skin diathesis. Otherwise, no remarkable differences were detected between *FLG* loss-of-function carriers and wild-type subjects. In addition, subgroup analysis to detect possible differences between compound heterozygous and heterozygous subjects did not show significant variations (detailed results not shown); however, it should be noted that the number of compound heterozygous carriers was low (n = 4). Regression analysis including *FLG* loss-of-function mutations and atopic skin diathesis as risk factors showed no significant impact of either atopic skin diathesis or *FLG* on type IV sensitization. The same was true regarding type IV polysensitization (Table 2).

Discussion

In this study, a well-defined cohort of patients with occupational contact dermatitis of the hands was investigated for possible associations between sensitization to common type I and IV allergens and the presence of *FLG* loss-of-function mutations.

Our results showed no statistically significant differences between the two groups regarding sensitization to common type I allergens, allergic rhinoconjunctivitis, or allergic asthma. *FLG* loss-of-function mutations were significantly associated with atopic skin diathesis. A tendency ($p = 0.06$) for a higher rate of polysensitization to type I allergens in *FLG* loss-of-function mutation carriers than in wild-type subjects (21.7% vs. 12.7%) was found in the unadjusted chi-square analysis. Polysensitization may be related to increased penetration of allergens resulting from a disrupted skin barrier or to the strong atopic background that was seen in *FLG* loss-of-function mutation carriers. This association seems to be relevant, as logistic regression analysis on type I sensitization and polysensitization showed a significant impact of atopic skin diathesis and no significant effect of *FLG* loss-of-function mutations.

The powerful association between *FLG* loss-of-function mutations and atopic skin diathesis might also – at least partly – account for the increased mean level of total IgE that was detected among *FLG* loss-of-function carriers. There is a consensus that atopic individuals have a tendency to develop IgE antibodies against environmental antigens. Earlier studies have also shown increased IgE levels in *FLG* loss-of-function mutation carriers in the context of AE (11–14). It has been speculated that genetically determined primary epidermal barrier dysfunction is the key event in AE that predisposes to secondary allergic manifestations, because of reduced resistance to allergens and irritants. In contrast, others did not find an association between serum IgE and *FLG* loss-of-function mutations (15, 16). *FLG* loss-of-function mutations have also been linked to other atopic phenotypes; in asthma, they conferred to a risk of up to 5.69, but this effect was limited to individuals with coexisting AE (11, 14, 17–22). As filaggrin is not expressed in the respiratory epithelium of the nose or lower airways, it has been suggested that *FLG*-associated asthma is mediated through percutaneous priming or secondary immunological mechanisms stimulated through the impaired skin barrier (23). However, in subgroups of patients without AE, *FLG* loss-of-function mutations showed no association with asthma (17, 21, 24). Comparable positive associations with *FLG* loss-of-function mutations were also reported for allergic rhinoconjunctivitis, but, again, this was limited to patients with coexisting AE (11, 17). Only one study reported an eczema-independent association of *FLG* loss-of-function mutations and allergic rhinoconjunctivitis (OR 2.64, 95% CI 1.76–4.00) (18). As the majority of studies on type I allergies were conducted in children with a high proportion of coexisting AE, results have to be

Table 2. Regression analysis including *FLG* loss-of-function mutations and atopic skin diathesis as risk factors for at least one type I (IV) sensitization and type I (IV) polysensitization

	Total number of tested	Risk factors			
		<i>FLG</i> loss-of-function mutation		Atopic skin diathesis	
		OR	95% CI	OR	95% CI
At least one type I sensitization	431	0.84	0.48–1.45	1.58	1.04–2.41
Type I polysensitization	431	1.72	0.86–3.44	2.15	1.05–4.41
At least one type IV sensitization	496	0.94	0.54–1.61	1.45	0.97–2.18
Type IV polysensitization	496	0.78	0.35–1.72	1.40	0.77–2.55

CI, confidence interval; OR, odds ratio.

Significant results are shown in bold.

interpreted and compared with caution, as they may not be representative for other age groups, such as adults. Similar to our results, a large cross-sectional population-based study on adult subjects from Denmark was not able to detect significant associations between the two most common *FLG* loss-of-function mutations, R501X and 2282del4, and allergic rhinitis and asthma respectively (16).

In our study, prick testing did not show significant differences in rates of sensitization to the most common allergens between wild-type subjects and *FLG* loss-of-function mutation carriers. As reported by Henderson, prick testing on children showed strong associations between *FLG* loss-of-function mutations and specific sensitization to grass, house dust mite, and cat dander, and sensitization to multiple allergens (OR 2.12, 95% CI 1.03–4.37), in the presence of AE (17), but not in the absence of AE. For natural rubber latex, a common occupational type I allergen, we did not find associations with *FLG* loss-of-function mutations, which is in accordance with a study on occupationally exposed dentists and dental personal by Carlsen et al. (25).

Regarding type IV sensitization, some authors have suggested decreased cell-mediated immunity for atopic individuals (26), which would lead to the observation of decreased rates of allergic contact dermatitis. This assumption was derived from early investigations in which patients with severe AE were not readily sensitized by repeated application of dinitrochlorobenzene (DNCB) in experimental settings. However, once the eczema has improved or in periods of remission, the DNCB challenge became positive (26, 27). More recent studies have shown that atopic individuals are at least as likely to develop type IV sensitization as non-atopics (28, 29). Several reasons may account for this finding: (i) atopic individuals may have an increased incidence of allergic contact sensitization; (ii) they have a higher degree of exposure to topical medications and moisturizers, because of their disease; and (iii) the disruption of

their skin barrier permits increased penetration of allergens.

Similarly to our results, Carlsen et al. did not find associations between the *FLG* loss-of-function mutations R501X and 2282del4 and sensitization to allergens from the European baseline patch test series (30). This group hypothesized that, because contact allergens usually have a molecular weight of <500, and protein allergens are much larger, the increased allergen penetration through filaggrin-deficient skin may only concern protein allergens, and contact allergens may penetrate the epidermis regardless of *FLG* mutation status, because of their small size; this may hold true for our findings regarding type IV sensitization (30).

In our study, only sensitization to two type IV allergens showed statistically significant differences concerning the presence of *FLG* loss-of-function mutations as a risk factor, with higher sensitization rates for lanolin and PTBP-FR. Lanolin (wool alcohols) has been a vehicle for skin care creams and ointments for decades. Frequent sensitization to lanolin has been associated with stasis dermatitis, leg ulcers, and AE (29, 31). Because *FLG* loss-of-function mutation carriers have dry and inflamed skin, their use of topical agents containing lanolin may be increased, and this finding is most likely related to special treatment exposure. PTBP-FR hypersensitivity may result from either to the *p*-tert-butylphenol, from the resin itself, or, less frequently, from the formaldehyde (32). These resins often cause occupational allergy. As they are mainly used in adhesives and paints, employees from the shoe-making and automobile industries are frequently affected. Although this allergy was found more often in *FLG* loss-of-function mutation carriers, the CI is large (detailed results not shown), and the results may be incidental.

Regarding nickel sulfate, an allergen that has been extensively investigated in the context of *FLG* loss-of-function mutations, we observed no significant association. Novak et al. described a positive association between contact sensitization to nickel in combination

Table 3. Rates of sensitization to common allergens from the European baseline patch test series in wild-type subjects and *FLG* loss-of-function mutation carriers

Allergen	Test concentration % ^a	<i>FLG</i> wild-type subjects, n = 429 n (%)	<i>FLG</i> loss-of function mutation carriers, n = 67 n (%)
Potassium dichromate	0.5	51 (11.9)	6 (9)
Thiuram mix	1	46 (10.7)	9 (13.4)
Cobalt chloride	1	56 (13.1)	11 (16.4)
<i>Myroxylon pereirae</i>	25	40 (9.3)	6 (9)
Colophonium	20	26 (6.1)	1 (1.5)
<i>N</i> -isopropyl- <i>N</i> -phenyl- <i>p</i> -phenylenediamine	0.1	10 (2.3)	1 (1.5)
Lanolin (wool alcohols)	30	9 (2.1)	6 (9)**
Mercapto mix	1	18 (4.2)	4 (6)
Epoxy resin	1	14 (3.3)	0
Nickel sulfate	5	121 (28.2)	13 (19.4)
<i>p</i> -tert-Butylphenol-formaldehyde resin	1	3 (0.7)	3 (4.5)*
Formaldehyde	1, aqua	17 (4)	0
Fragrance mix I	8	47 (11)	7 (10.4)
Oil of turpentine	10	11 (2.6)	4 (6)
Methylchloroisothiazolinone/methylisothiazolinone	100 ppm, aqua	24 (5.6)	3 (4.5)
Paraben mix	16	26 (6.1)	2 (3.0)
Cetearyl alcohol	20	4 (0.9)	0
Zinc diethyldithiocarbamate	1	2 (0.5)	0
Methyldibromo glutaronitrile	0.2	47 (11)	7 (10.4)
Propolis	10	12 (2.8)	2 (3)
Bufexamac	5	24 (5.6)	6 (9)
Compositae mix	5	19 (4.4)	1 (1.5)
Mercaptobenzothiazole	2	18 (4.2)	4 (6)
Hydroxyisohexyl 3-cyclohexene carboxaldehyde	5	17 (4)	2 (3)
2-Bromo-2-nitropropane-1,3-diol	0.5	9 (2.1)	3 (4.5)
Fragrance mix II	14	17 (4)	4 (6)
Ylang-ylang oil	10	3 (0.7)	1 (1.5)
Sandalwood oil	10	0	0
<i>Jasminum officinale</i>	5	2 (0.5)	0
At least one type IV sensitization		276 (64.3)	43 (64.2)
Polysensitization (sensitization to three or more allergens)		61 (14.2)	8 (11.9)

*Significant difference at $p < 0.05$. **Significant difference at $p < 0.01$.

Significant results are shown in bold.

^a Vehicle: petrolatum unless stated otherwise.

with intolerance to fashion jewellery and *FLG* loss-of-function mutation carriers (33). Ross-Hansen reported an increasing proportion of *FLG* loss-of-function mutation carriers with stronger patch test reactions to nickel (34). Thyssen et al. found no association between *FLG* loss-of-function mutations and contact sensitization to nickel in the overall population. However, when a subgroup analysis was performed on females without ear piercing, a trend for a positive but not statistically significant association was seen (16). Similarly, Carlsen et al. described an overrepresentation of positive patch test reactions to nickel among *FLG* loss-of-function mutation carriers (*FLG* 45.9% versus wild type 34%), although the results did not reach the level of significance (30). It has

been speculated that different routes of exposure may be fundamental to nickel sensitization, because filaggrin, as a histidine-rich protein in the upper dermis, could prevent its skin penetration by chelating it (35).

Limitations

The following limitations of this study need to be recognized. First, our study sample was drawn from selected patients who were referred to a tertiary referral centre with long-lasting chronic contact dermatitis of the hands; therefore, patients with atopy and AE may be overrepresented, and the results may not be characteristic of the general population. Second, larger cohorts may

be necessary to increase the statistical power of the findings presented, particularly regarding type I and IV sensitization.

Conclusions

The results showed no remarkable differences in the prevalence rates of sensitization to common type I and IV allergens between *FLG* loss-of-function mutation carriers and wild-type subjects.

Higher rates of polysensitization to type I allergens for *FLG* loss-of-function mutation carriers were observed, which is most probably related to the strong atopic background that was seen in these subjects. The increased prevalence of type IV sensitization to lanolin in *FLG* loss-of-function mutation carriers is most likely related

to the increased use of this to treat dry and inflamed skin, which is commonly seen in subjects with filaggrin deficiency, particularly in those with coexisting AE. It can be speculated that contact allergens, because of their small size, may penetrate the epidermis regardless of *FLG* loss-of-function mutation status, and only for larger protein allergens may filaggrin-deficient skin allow penetration to a greater degree, owing to impaired skin barrier function. Currently, the significance of filaggrin deficiency in allergic sensitization, particularly in the absence of AE, remains incompletely understood. Probably a variety of immunological mechanisms other than that resulting from the filaggrin system modulate allergic sensitization to a greater degree. Large population-based studies are needed to gain further insights.

References

- Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med* 2011; **365**: 1315–1327.
- Visser MJ, Landeck L, Campbell LE, McLean WH, Weidinger S, Calkoen F, John SM, Kezic S. Impact of loss-of-function mutations in the filaggrin gene and atopic dermatitis on the development of occupational irritant contact dermatitis. *Br J Dermatol* 2013; **168**: 326–332.
- Landeck L, Visser M, Skudlik C, Brans R, Kezic S, John SM. Clinical course of occupational irritant contact dermatitis of the hands in relation to filaggrin genotype status and atopy. *Br J Dermatol* 2012; **167**: 1302–1309.
- Scharschmidt TC, Man MQ, Hatano Y et al. Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. *J Allergy Clin Immunol* 2009; **124**: 496–506.
- Fallon PG, Sasaki T, Sandilands A et al. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. *Nat Genet* 2009; **41**: 602–608.
- Diepgen TL, Sauerbrei W, Fartasch M. Development and validation of diagnostic scores for atopic dermatitis incorporating criteria of data quality and practical usefulness. *J Clin Epidemiol* 1996; **49**: 1031–1038.
- Landeck L, Visser M, Kezic S, John SM. Genotype–phenotype associations in filaggrin loss-of-function mutation carriers. *Contact Dermatitis* 2013; **68**: 149–155.
- Rüeff F, Bergmann K C, Brockow K et al. Skin tests for diagnostics of allergic immediate-type reactions. Guideline of the German Society for Allergology and Clinical Immunology. *Pneumologie* 2011; **65**: 484–495.
- Lindberg M, Matura M. Patch testing. In: *Textbook of Contact Dermatitis*, Johansen J D, Frosch P J, Leppoittevin J P (eds): Berlin, Springer, 2011; pp. 439–464.
- Carlsen B C, Andersen K E, Menné T, Johansen J D. Patients with multiple contact allergies: a review. *Contact Dermatitis* 2008; **58**: 1–8.
- Ekelund E, Liedén A, Link J, Lee S P, D'Amato M, Palmer C N, Kockum I, Bradley M. Loss-of-function variants of the filaggrin gene are associated with AE and associated phenotypes in Swedish families. *Acta Derm Venereol* 2008; **88**: 15–19.
- Marenholz I, Nickel R, Rüschendorf F et al. Filaggrin loss-of-function mutations predispose to phenotypes involved in the atopic march. *J Allergy Clin Immunol* 2006; **118**: 866–871.
- Weidinger S, Illig T, Baurecht H et al. Loss-of-function variations within the filaggrin gene predispose for atopic dermatitis with allergic sensitizations. *J Allergy Clin Immunol* 2006; **118**: 214–219.
- Weidinger S, Rodríguez E, Stahl C, Wagenpfeil S, Klopp N, Illig T, Novak N. Filaggrin mutations strongly predispose to early-onset and extrinsic atopic dermatitis. *J Invest Dermatol* 2007; **127**: 724–726.
- O'Regan G M, Kemperman P M, Sandilands A et al. Raman profiles of the stratum corneum define 3 filaggrin genotype-determined atopic dermatitis endophenotypes. *J Allergy Clin Immunol* 2010; **126**: 574–580.
- Thyssen J P, Johansen J D, Linneberg A et al. The association between null mutations in the filaggrin gene and contact sensitization to nickel and other chemicals in the general population. *Br J Dermatol* 2010; **162**: 1278–1285.
- Henderson J, Northstone K, Lee S P et al. The burden of disease associated with filaggrin mutations: a population-based, longitudinal birth cohort study. *J Allergy Clin Immunol* 2008; **121**: 872–877.
- Weidinger S, O'Sullivan M, Illig T et al. Filaggrin mutations, AE, hay fever, and asthma in children. *J Allergy Clin Immunol* 2008; **121**: 1203–1209.
- van den Oord R A, Sheikh A. Filaggrin gene defects and risk of developing allergic sensitization and allergic disorders: systematic review and meta-analysis. *BMJ* 2009; **339**: b2433.
- Morar N, Cookson W O, Harper J I, Moffatt M F. Filaggrin mutations in children with severe atopic dermatitis. *J Invest Dermatol* 2007; **127**: 1667–1672.
- Brown S J, Relton C L, Liao H et al. Filaggrin null mutations and childhood AE: a population-based case–control study. *J Allergy Clin Immunol* 2008; **121**: 940–946.
- Osawa R, Akiyama M, Shimizu H. Filaggrin gene defects and the risk of developing allergic disorders. *Allergol Int* 2011; **60**: 1–9.
- Ying S, Meng Q, Corrigan C J, Lee T H. Lack of filaggrin expression in the human bronchial mucosa. *J Allergy Clin Immunol* 2006; **118**: 1386–1388.

- 24 Rodríguez E, Baurecht H, Herberich E, Wagenpfeil S, Brown S J, Cordell H J, Irvine A D, Weidinger S. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. *J Allergy Clin Immunol* 2009; **123**: 1361–1370.
- 25 Carlsen B C, Meldgaard M, Hamann D et al. Latex allergy and filaggrin null mutations. *J Dent* 2011; **39**: 128–132.
- 26 Rogge J L, Hanifin J M. Immunodeficiencies in severe atopic dermatitis. Depressed chemotaxis and lymphocyte transformation. *Arch Dermatol* 1976; **112**: 1391–1396.
- 27 Uehara M, Sawai T. A longitudinal study of contact sensitivity in patients with atopic dermatitis. *Arch Dermatol* 1989; **125**: 366–368.
- 28 Heine G, Schnuch A, Uter W, Worm M, Information Network of Departments of Dermatology (IVDK), German Contact Dermatitis Research Group (DKG). Type-IV sensitization profile of individuals with AE: results from the Information Network of Departments of Dermatology (IVDK) and the German Contact Dermatitis Research Group (DKG). *Allergy* 2006; **61**: 611–616.
- 29 Landeck L, Schalock P, Baden L, González E. Contact sensitization pattern in 172 atopic subjects. *Int J Dermatol* 2011; **50**: 806–810.
- 30 Carlsen B C, Johansen J D, Menné T, Meldgaard M, Szecei P B, Stender S, Thyssen J P. Filaggrin null mutations and association with contact allergy and allergic contact dermatitis: results from a tertiary dermatology clinic. *Contact Dermatitis* 2010; **63**: 89–95.
- 31 Wakelin S H, Smith H, White I R, Rycroft R J, McFadden J P. A retrospective analysis of contact allergy to lanolin. *Br J Dermatol* 2001; **145**: 28–31.
- 32 Foussereau J, Cavelier C, Selig D. Occupational eczema from para-tertiary-butylphenol formaldehyde resins: a review of the sensitizing resins. *Contact Dermatitis* 1976; **2**: 254–258.
- 33 Novak N, Baurecht H, Schäfer T et al. Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. *J Invest Dermatol* 2008; **128**: 1430–1435.
- 34 Ross-Hansen K, Menné T, Johansen J D et al. Nickel reactivity and filaggrin null mutations – evaluation of the filaggrin bypass theory in a general population. *Contact Dermatitis* 2011; **64**: 24–31.
- 35 Thyssen J P, Carlsen B C, Menné T. Nickel sensitization, hand eczema, and loss-of-function mutations in the filaggrin gene. *Dermatitis* 2008; **19**: 303–307.