

Genotype–phenotype associations in filaggrin loss-of-function mutation carriers

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Summary

Background. Loss-of-function mutations in the filaggrin gene (*FLG*) have been reported to be associated with specific phenotypic characteristics such as hyperlinearity and keratosis pilaris.

Objectives. To study phenotypic features in patients with occupational irritant contact eczema of the hands in relation to *FLG* loss-of-function mutations.

Materials and methods. In a prospective cohort study, genotype was determined for 459 study subjects for four *FLG* null alleles, and investigated for selected history, clinical and laboratory features.

Results. Overall, 68 patients showed a mutation in the *FLG* alleles R501X, R2447X, S3247X, and/or 2282del4. Flexural eczema, xerosis cutis, pityriasis alba, dirty neck, pulpitis sicca, hyperlinear palms, keratosis pilaris and family history of eczema were positively associated with *FLG* mutations ($p < 0.05$). Although we observed a statistically significant correlation with higher serum IgE in *FLG* mutation carriers, allergic rhinoconjunctivitis and allergic asthma were not over-represented in this group.

Conclusion. This study shows further genotype–phenotype correlations in patients with occupational irritant contact eczema and *FLG* mutation carrier status. These features may help to identify those with *FLG* mutations on a specific phenotype basis.

Key words: filaggrin loss-of-function mutation; genotype; irritant contact eczema; phenotype.

Dermatological diseases are increasingly being investigated on a molecular basis, often with prognostic implications. So far, more than 300 dermatological entities have been classified at a molecular level (1). Loss-of-function mutations in the filaggrin gene (*FLG*) have been identified as important for the development of atopic eczema. The presence of an *FLG* null allele correlates with a 1.2-fold to

1.3-fold higher risk of developing atopic eczema. Between 14% and 56% of individuals suffering from atopic eczema are carriers of at least one *FLG* mutation (2). Five loss-of-function mutations are highly prevalent within European Caucasians, giving a combined null allele frequency of 0.09, which is consistent with the finding that ~9% of the population are at a higher risk of developing atopic eczema (2).

In a case–control study, de Jongh et al. investigated the prevalence of *FLG* loss-of-function mutations R501X and 2282del4 in patients with chronic irritant contact eczema and in controls; 12.5% of patients and 6.9% of controls were found to be heterozygous for the null alleles R501X and 2282del4, translating to an odds ratio (OR) of 1.91 [95% confidence interval (CI) 1.02–3.59] (3). The authors suggested an increased susceptibility to

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chronic irritant contact eczema among *FLG* mutation carriers (3). Filaggrin has complex functions within the epidermis, constituting the structural and chemical barrier, and maintaining hydration and homeostasis. It aggregates keratin filaments in the stratum corneum, and is responsible for the mechanical strength of the skin. Filaggrin is the main source of hygroscopic constituents, and has a central role in sustaining hydration of the stratum corneum. One of its breakdown products, urocanic acid, has a significant role in maintenance of the pH gradient of the skin, cutaneous antimicrobial defence, and regulation of key enzymatic events (4). Consequently, it is likely that individuals with reduced levels of filaggrin will have an altered skin barrier and therefore be more susceptible to develop contact eczema.

Genetic counselling is not possible in all areas of patient care, and, in many countries, genetic investigations are prohibited in specific instances, such as pre-employment counselling (5). For those starting vocational training, especially if the work will be in a high-risk occupation with increased exposure to irritants, or if endogenous risk factors, for example atopic eczema and allergies, are prevalent in the family, it might be relevant for appropriate medical advice to be obtained before the start of training. Additionally, for patients who have already developed eczema, early-stage identification of those with genetic alterations such as *FLG* loss-of-function mutations may result in additional emphasis of the importance of adherence to specific therapeutic interventions and preventive measures to improve dermatological rehabilitation. Thus, genotype–phenotype correlations are important. The goal of this study was to investigate the phenotype in patients with irritant contact eczema of the hands according to *FLG* loss-of-function mutation carrier status.

Methods

Study population

After approval had been obtained from the ethics committee of the University of Osnabrück, a cohort of 712 patients who were hospitalized at our clinic for treatment of occupational hand eczema were asked to participate in the study, which was conducted in accordance with the Declaration of Helsinki. Forty-seven patients chose not to enrol. After informed consent had been obtained, genotype was determined for *FLG* 2282del4, R501X, R2447X and S3247X mutations between November 2005 and March 2011. Patients were consecutively included in this study if they met

the following inclusion criteria: (i) Caucasian, (ii) age ≥ 18 years, (iii) primary diagnosis of ICE of the hands, and (iv) no history of a secondary chronic inflammatory disorder (e.g. rheumatoid arthritis or psoriasis). Two hundred and six patients were excluded because of a primary diagnosis of allergic contact dermatitis or atopic eczema, or because of failure of filaggrin determination, leaving a total number of 459 patients for analysis. The diagnosis of ICE was based on the patient's history, exposure to irritants, clinical distribution and presence of skin lesions, exclusion of other dermatological entities, and absence of clinically relevant type IV sensitization. For each patient, an experienced dermatologist performed a full examination of the skin, and a detailed medical history was recorded with a standardized questionnaire. *FLG* mutation carriers were contrasted with wild-type subjects with regard to 23 clinical features and total IgE (Table 1).

In addition, the presence of 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). The Erlangen score is based on history and clinical atopic features as well as laboratory investigations (IgE).

Filaggrin genotyping

DNA material was obtained from buccal mucosa cells with buccal swabs (Medispo, Oud-Beijerland, The Netherlands). For each subject, two swabs were obtained, and 2 ml of lysis buffer (Puregene® Cell Lysis Solution; Gentra Systems, Minneapolis, MN, USA) was added with each swab to disrupt the cells and stabilize the DNA. Extraction and genotyping for the mutations R501X, R2447X and S3247X were performed by KBioscience (<http://www.kbioscience.co.uk>). Mutations were genotyped with the KASP genotyping system, a homogeneous fluorescence resonance energy transfer-based system, coupled with competitive allele-specific polymerase chain reaction. 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 GCACTGGAGGAAGACAAGGATCG. R2447X was genotyped by using the primer pair GAGTGCCTGGAGCTGTCTCG (C-allele) and GAGTGCCTGGAGCTGTCTCA (T-allele) with the common allele primer GAGGAAGACAAGGATCCCACACACA. S3247X was genotyped by using the primer pair GTGTCTGGAGCCGTGCCTTG (C-allele) and GGTGTCTGGAGCCGTGCCTTT (A-allele) with the common primer CTTCCAGAAACCATCGTGGATCTGT. Genotyping for 2282del4 was performed as described previously (7). Analyses of the mutations were performed in

Table 1. Clinical features and total IgE in irritant contact eczema patients with and without *FLG* mutations

Clinical feature	Total, n	WT total	WT Yes (n)	WT Yes (%)	<i>FLG</i> total	<i>FLG</i> Yes (n)	<i>FLG</i> Yes (%)	p-value	OR	95% CI	Sensitivity (%)	Specificity (%)
Eczema in family history (first-degree)	455	387	136	35.1	68	35	51.5	0.01	1.96	1.12–3.30	51.5	64.9
<i>Rhinitis/asthma in family history</i> (first-degree)	457	389	149	38.3	68	27	39.7	0.89	1.06	0.63–1.80	39.7	61.7
Flexural eczema (skin rash affecting the flexural areas)	455	388	142	36.6	67	42	62.7	0	2.91	1.70–5.00	62.7	63.4
<i>Rhinoconjunctivitis</i> (tearing, redness of eyes and nose, stuffed and runny nose, itching, sneezing)	453	385	145	37.7	68	28	41.2	0.59	1.16	0.69–1.96	41.2	62.3
<i>Allergic asthma</i> (recurrent bouts of wheezing and coughing and spastic respiratory symptoms without physical exertion)	454	386	76	19.7	68	18	26.5	0.20	1.47	0.81–2.66	26.5	80.3
<i>Cradle cap</i> (scaling and crusting of the scalp during infancy)	404	343	44	12.8	61	11	18	0.31	1.50	0.72–3.10	18	87.2
<i>Itch when sweating</i>	457	389	116	29.8	68	23	33.8	0.57	1.20	0.70–2.08	33.8	70.2
<i>Wool intolerance</i> (discomfort, irritation, itching when wearing woollen textiles next to the skin)	458	390	137	35.1	68	26	38.2	0.68	1.14	0.62–1.95	38.2	64.9
<i>Metal intolerance</i> (itching and skin rash when contact with metals, especially nickel, occurs)	455	388	141	36.3	67	17	25.4	0.09	0.60	0.33–1.07	25.4	63.7
<i>Photophobia</i> (direct sunlight induces itchy eyes and running tears)	459	391	72	18.4	68	15	22.1	0.50	1.25	0.67–2.35	22.1	81.6
Xerosis cutis (generalized dry skin)	458	390	277	71	68	62	91.2	0	4.22	1.77–10.02	91.2	29
<i>Ear rhagades</i> (any eczema around the ears)	459	391	64	16.4	68	13	19.1	0.6	1.21	0.62–2.34	19.1	83.6
<i>Dyshidrosis</i> (recurring itchy vesicles at the interdigital or thenar region of the hands)	456	388	249	64.2	68	47	69.1	0.49	1.25	0.72–2.18	69.1	35.8
Pityriasis alba (patchy hypopigmentation, mostly on sun-exposed areas of uninvolved skin)	457	389	26	6.7	68	10	14.7	0.046	2.41	1.10–5.25	14.7	93.3
Pulpitis sicca (cracked fingertips)	459	391	46	11.8	68	17	25	0.007	2.5	1.33–4.69	25	88.2
<i>Nipple eczema</i> (skin rash, itching and inflammation around the nipples)	459	391	4	1.0	68	1	1.5	0.553	1.44	0.16–13.12	1.5	99
<i>Perlèche</i> (rhagades, erythema, and painful dermatitis at the angles of the mouth)	459	391	84	21.5	68	20	29.4	0.159	1.52	0.86–2.71	29.4	78.5

Table 1. *Continued*

Clinical feature	Total, n	WT total	WT Yes (n) Yes (%)	FLG total	FLG Yes (n) Yes (%)	p-value	OR	95% CI	Sensitivity (%)	Specificity (%)
Hyperlinear palms (prominent lines across the thenar and hypothenar eminences)	459	391	191 48.8	68	45 66.2	0.009	2.05	1.19–3.52	66.2	51.2
<i>Hertoghe sign</i> (thinning or complete absence of the lateral aspects of the eyebrows)	459	391	65 16.6	68	16 23.5	0.17	1.54	0.83–2.87	23.5	83.4
Dirty neck (rippled pattern of hyperpigmentation at the neck)	459	391	8 2	68	8 11.8	0.001	6.38	2.31–17.65	11.8	98
Keratosis pilaris (horny follicular papules involving posterolateral aspects of upper arms, thighs, or buttocks)	419	359	81 22.6	60	22 36.7	0.023	1.99	1.11–3.55	37	77
<i>White dermographism</i> (mechanical stroking of uninvolved skin at the infrascapular region results in a white line instead of the normal reddish response)	458	390	166 42.6	68	33 48.5	0.43	1.27	0.76–2.13	48	57
<i>Acrocyanosis</i> (persistent blue discoloration of the hands, feet, and/or distal parts of the face, ears, nose, which is usually not accompanied by pain and/or trophic skin changes)	457	389	39 10	68	12 17.6	0.092	1.92	0.95–3.90	17.6	90
Total serum IgE*	95	79	321	16	991	0				

Bold, significant findings; CI, confidence interval; OR, odds ratio; WT, wild-type carriers.

*Mean value in kU/l; raised total IgE was taken to be > 100 kU/l.

a blinded fashion; laboratory staff did not have access to the clinical data or the phenotypes of the patients. Conversely, dermatologists performing full skin examination and recording the medical history did not know the *FLG* status of the patients.

Data analyses and statistics

The observed genotype frequencies were compared with the expected Hardy–Weinberg distribution by use of the chi-square test. To estimate the risk of a clinical feature conferred by a particular genotype, we calculated the ORs with 95% CIs of a subject's genotype, comparing the heterozygous and homozygous variant allele genotypes with the homozygous wild-type genotype by use of the additive model. Patients with incidental missing data were excluded from the statistical analysis of that specific variable. The statistical analyses were performed with SPSS™ version 16.0 (SPSS, Chicago, IL, USA).

Results

Our study included 459 patients. The tested individuals were aged 18–67 years (mean 41 ± 12 years) in this cohort of 279 (60.7%) females and 180 males. One hundred and thirty-two patients (28.8%) were diagnosed with ICE without atopic skin diathesis, and the remaining 327 (71.2%) with ICE with atopic skin diathesis. The genotyping success rates were 98.6% for 2282del4, 97.7% for R501X, 95.9% for R2447X, and 96.7% for S3247X. Of the total study population, 68 (14.8%) individuals were carriers of an *FLG* mutation (ICE, $n = 8$; ICE with atopic skin diathesis, $n = 60$). Among these, we identified 5 compound heterozygotes (carriage of more than one *FLG* mutation) and 63 heterozygotes. No homozygote carriers were seen.

On comparison of *FLG* loss-of-function and wild-type individuals, nine clinical features yielded significant positive associations with *FLG* mutation status: flexural eczema, xerosis cutis, pityriasis alba, dirty neck, pulpitis sicca, hyperlinearity of the palms, keratosis pilaris, eczema in family history, and elevated serum IgE level (Table 1). A trend for a negative association was seen for metal intolerance, occurring less often in the *FLG* mutation group. However, the difference did not reach the level of significance of $p < 0.05$.

The strongest significant positive associations were seen for the dirty neck sign (OR 6.38, 95% CI 2.31–17.65, $p = 0.001$), xerosis cutis (OR 4.22, 95% CI 1.77–10.02, $p = 0$), and flexural eczema (OR 2.91, 95% CI 1.7–5.0, $p = 0$).

The highest rates of sensitivity were found for xerosis cutis (91.2%), dyshidrosis (69.1%), hyperlinear palms

(66.2%), and flexural eczema (62.7%), meaning that these clinical features showed the highest percentages of patients who were correctly identified as having the mutation. The absence of nipple eczema, of the dirty neck sign and pityriasis alba were reliable denominators for the detection of wild-type subjects, giving the highest rates for specificity, as shown in Table 1.

Discussion

Hyperlinearity of the palms, asthma and keratosis pilaris are the phenotypic characteristics most commonly mentioned in the literature as being associated with *FLG* null mutations, particularly in the presence of atopic eczema (8–10). Our study aimed to investigate phenotypic markers in a subgroup of individuals with *FLG* null mutations in a well-defined cohort of patients with ICE of the hands. All of our 459 patients were carefully interviewed and examined by experienced dermatologists. The findings confirmed well-known phenotypic *FLG* associations, but also showed interesting new associations.

The results of our study corroborated the well-recognized phenotypic characteristics of keratosis pilaris and hyperlinearity, showing two-fold higher ORs for *FLG* mutation carriers. Both clinical signs have shown independent strong associations with *FLG* null mutations (8, 11, 12). Palmar hyperlinearity was found to have a positive predictive value of 71% (10), whereas the absence of both hyperlinearity and keratosis pilaris was shown to have a negative predictive value of 92%, meaning that 92% of patients without these features do not carry *FLG* null mutations (10).

The strongest significant correlation was seen for the 'dirty neck sign', which is characterized by a rippled pattern of hyperpigmentation of the neck. The risk of developing a dirty neck was increased more than six-fold in *FLG* mutation carriers as compared with wild-type subjects. To our knowledge, such an association has not yet been described. Further strong genotype–phenotype associations were detected for xerosis cutis (dry skin) and flexural eczema, translating to a four-fold and three-fold higher risk, respectively, in *FLG* mutation carriers. This is in accordance with a study by Novak et al., who found an OR of 2.95 for dry skin in carriers of the combined null mutation R501X and 2282del4 (13). O'Regan et al. assessed the composition and function of the stratum corneum in moderate-to-severe atopic eczema patients with and without *FLG* null mutations. Low levels of the natural moisturizing factor, which plays a central role in maintaining stratum corneum hydration, were correlated with *FLG* null mutations, suggesting a direct contribution to the dry skin phenotype seen in these patients (8).

Similar associations between decreased natural moisturizing factor levels and *FLG* loss-of-function mutations were described by Mlitz et al. (14). Flohr investigated 88 infants, and found that carriers of *FLG* mutations independent of atopic eczema were significantly more likely to have clinically dry skin (OR 8.5) (15). Jungersted et al. studied biophysical parameters related to atopy and *FLG* status. Transepidermal water loss (TEWL) resulting in skin that was more prone to xerosis was generally higher in *FLG* mutation carriers, independently of additional atopic eczema status. A trend towards lower skin hydration values in atopic eczema patients, particularly those with *FLG* loss-of-function mutations, has been described (16). A similar study conducted by Angelova-Fischer et al. also described increased baseline TEWL in *FLG* mutation carriers with atopic eczema, and reduced levels of stratum corneum hydration, in contrast to healthy controls (17).

Regarding total serum IgE, we observed much higher levels in *FLG* loss-of-function mutation carriers than in wild-type subjects (321 versus 991 kU/l). In a family-based association study, Weidinger et al. found that *FLG* mutations predisposed to the extrinsic subtype of atopic eczema, which is characterized by high total serum IgE and concomitant allergic sensitization (11). A later study by this group investigated the same association between atopic eczema and *FLG* null mutations in 274 unrelated atopic eczema adults and 252 population-based controls. Again, both *FLG* variants R501X and 2282del4 showed significant associations with total serum IgE (*FLG* mutants 317 kU/l versus wild-type carriers 136 kU/l), and with asthma and rhinitis in the context of atopic eczema (18). The authors concluded that genetically determined primary epidermal barrier disruption is the key event in atopic eczema that predisposes to secondary allergic manifestations, because of reduced resistance to environmental agents such as allergens, irritants, and microbes (18). However, others did not find an association between total serum IgE and *FLG* mutations (8). Although the hypothesis of Weidinger et al. seems plausible, it was not applicable to our cohort. Despite significantly higher serum IgE levels among the *FLG* mutation group, 88% of whom had a strong atopic background in their medical history, allergic asthma and rhinoconjunctivitis were not significantly over-represented in our subgroup of *FLG* loss-of-function mutation carriers. This finding is in contrast to the majority of studies, which have shown relationships between *FLG* null mutations, rhinoconjunctivitis, and allergic asthma, particularly in the presence of atopic eczema. Henderson et al. analysed the impact of the null alleles R501X and 2282del4 on several atopic phenotypes in 7000 school children. *FLG* mutations conferred a population asthma risk of 1.80 (95% CI

1.34–2.41; $p = 0.00019$). In the context of eczema, even asthma risk was increased more than three-fold (OR 3.16; 95% CI 2.25–4.43, $p = 1.4 \times 10^{-11}$). However, in the absence of eczema, *FLG* alleles had no association with asthma (OR 0.80; 95% CI 0.46–1.41). Weaker positive associations in the presence of eczema were described for hay fever (19). Brown et al. investigated 811 English children, and found that asthma, but not seasonal rhinitis, in the context of atopic eczema showed a significant association with *FLG* mutations (12). A recent meta-analysis of 17 studies involving 3138 cases and 17 164 controls found significant associations between *FLG* mutations and asthma, independently of the presence of eczema (OR 1.48; 95% CI 1.32–1.66) (9). Weidinger et al. conducted a cross-sectional study, including 3099 children, on the effect of *FLG* mutations at the population level and their effect on other atopic phenotypes. Independently of eczema, *FLG* mutations conferred a substantial risk for allergic rhinitis (OR 2.64, 95% CI 1.76–4.00), whereas the association with asthma was restricted to the presence of eczema (OR 1.76, 95% CI 1.19–2.68) (20).

A trend for a negative association in *FLG* loss-of-function mutation carriers was seen for metal intolerance. Mainly, this characteristic refers to nickel allergy, as nickel is the most important metal allergen in patch test clinics. Because, theoretically, an impaired skin barrier, as seen in *FLG* carriers, may facilitate the penetration of allergens, one could assume an increased rate of allergic contact sensitization in *FLG* mutants. Investigations on this topic are contradictory, and most studies have not confirmed the hypothesis of a positive association of *FLG* mutations with contact allergy (21–23). However, Novak et al. described associations with nickel contact sensitization and intolerance to fashion jewellery (13). In addition, it was reported that subjects with *FLG* mutations do have stronger patch test reactivity to nickel than wild-type individuals (24). Thyssen et al. speculated that different routes of nickel exposure may influence the sensitization rates in *FLG* carriers (23). Filaggrin, a histidine-rich protein of the upper epidermis, could chelate nickel, preventing its penetration through the skin in cases of exposure related to superficial contact with, for example, metallic suspenders, which might lead to decreased rates of nickel sensitization. Conversely, in cases of direct nickel ion exposure to Langerhans cells without previous penetration, as seen in ear piercing, this 'preventive' effect could be cancelled (23).

Limitations

Although patients with a primary diagnosis of atopic eczema were excluded from this study, there might be an overlap in phenotypes, as subjects with an atopic diathesis will tend to develop ICE.

Conclusions

This study shows further genotype–phenotype correlations in patients with occupational ICE of the hands and *FLG* loss-of-function mutation carrier status. On comparison of *FLG* mutation and wild-type individuals, nine clinical features showed significant positive associations with *FLG*: flexural eczema, xerosis cutis, pityriasis alba, dirty neck, pulpitis sicca, hyperlinearity of the palms, keratosis pilaris, family history of eczema, and elevated serum

IgE level. A trend of a negative association was seen for metal intolerance. Clinical features may help to identify those with *FLG* mutations on a specific phenotype basis.

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References

- McGrath J A, Uitto J. The filaggrin story: novel insights into skin-barrier function and disease. *Trends Mol Med* 2008; **14**: 20–27.
- Brown S J, Irvine A D. Atopic eczema and the filaggrin story. *Semin Cutan Med Surg* 2008; **27**: 128–137.
- de Jongh C M, Khrenova L, Verberk M M, Calkoen F, van Dijk F J, Voss H, John S M, Kezic S. Loss-of-function polymorphisms in the filaggrin gene are associated with an increased susceptibility to chronic irritant contact dermatitis: a case-control study. *Br J Dermatol* 2008; **159**: 621–627.
- Gibbs N K, Tye J, Norval M. Recent advances in urocanic acid photochemistry, photobiology and photoimmunology. *Photochem Photobiol Sci* 2008; **7**: 655–667.
- Gendiagnostikgesetz-GenDG. vom 31. Juli 2009. Available at: <http://www.gesetze-im-internet.de/bundesrecht/genDG/gesamt.pdf> (last assessed 5 June 2012).
- Diepgen T L, 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.
- Palmer C N, Irvine A D, Terron-Kwiatkowski A et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006; **38**: 441–446.
- 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.e1.
- 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.e7.
- Brown S J, Relton C L, Liao H et al. Filaggrin mutations in atopic eczema: genotype–phenotype correlation. *Br J Dermatol* 2008; **159** (Suppl 1): 11.
- 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.
- Brown S J, Relton C L, Liao H et al. Filaggrin null mutations and childhood atopic eczema: a population-based case-control study. *J Allergy Clin Immunol* 2008; **121**: 940–946.
- 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.
- Mlitz V, Latreille J, Gardinier S et al. Impact of filaggrin mutations on Raman spectra and biophysical properties of the stratum corneum in mild to moderate atopic dermatitis. *J Eur Acad Dermatol Venereol* 2012; **26**: 983–990.
- Flohr C, England K, Radulovic S, McLean W H, Campbell L E, Barker J, Perkin M, Lack G. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. *Br J Dermatol* 2010; **163**: 1333–1336.
- Jungersted J M, Scheer H, Mempel M et al. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. 2010; **65**: 911–918.
- Angelova-Fischer I, Mannheimer A C, Hinder A, Ruether A, Franke A, Neubert R H, Fischer T W, Zillikens D. Distinct barrier integrity phenotypes in filaggrin-related atopic eczema following sequential tape stripping and lipid profiling. *Exp Dermatol* 2011; **20**: 351–356.
- 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.
- 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**: 872–887.e9.
- Weidinger S, O'Sullivan M, Illig T et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol* 2008; **121**: 1203–1209.
- Carlsen B C, Thyssen J P, Menné T et al. Association between filaggrin null mutations and concomitant atopic dermatitis and contact allergy. *Clin Exp Dermatol* 2011; **36**: 467–472.
- Lerbaek A, Bisgaard H, Agner T, Ohm Kyvik K, Palmer C N, Menné T. Filaggrin null alleles are not associated with hand eczema or contact allergy. *Br J Dermatol* 2007; **157**: 1199–1204.
- 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.
- 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.