

Filaggrin null mutations increase the risk and persistence of hand eczema in subjects with atopic dermatitis: results from a general population study

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Summary

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Conflict of interest

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Background Hand eczema is prevalent in the general population. It remains unclear whether or not filaggrin gene (FLG) null mutations increase the overall risk of hand eczema or only increase the risk of hand eczema in subjects with atopic dermatitis.

Objectives To investigate the association between FLG null mutations and hand eczema.

Methods A random sample of 3335 adults from the general population in Denmark was patch tested, FLG genotyped for R501X and 2282del4 null mutations and questioned about hand eczema.

Results Participants with combined presence of atopic dermatitis and FLG null mutation status had a significantly higher prevalence of hand eczema, an earlier onset of hand eczema and a higher persistence of hand eczema compared with subjects with normal FLG status and absence of atopic dermatitis. Logistic regression analyses revealed positive associations between hand eczema within the past 12 months and FLG null mutation status in participants with a history of atopic dermatitis [odds ratio (OR) 2.98; 95% confidence interval (CI) 1.27–7.01], but not in subjects without atopic dermatitis (OR 0.82; 95% CI 0.41–1.67).

Conclusions FLG null mutations were significantly associated with hand eczema (< 12 months) in subjects with atopic dermatitis. Combined atopic dermatitis and filaggrin null mutation status was strongly associated with early onset of hand eczema and hand eczema persistence.

Hand eczema is prevalent in the general population.¹ A recent review, based on studies performed between 1964 and 2007, estimated that the point prevalence of hand eczema was around 4%, the 1-year prevalence nearly 10%, and the lifetime prevalence around 15%.¹ A high incidence rate of hand eczema was associated with female sex, atopic dermatitis, contact allergy and wet work. In fact, atopic dermatitis appeared to be the single most important risk factor for hand eczema.^{2–8} A Danish twin study found that hereditary factors were associated with hand eczema, independent of atopic dermatitis.^{2,9} Lerbaek et al.¹⁰ challenged this finding by investigating whether or not null mutations in the filaggrin gene (FLG) complex were associated with hand eczema. They found no statistically significant association in 183 twins, perhaps due

to low statistical power as well as an unfavourable control group.^{10,11} Nevertheless, they concluded that FLG null mutation status could be used as a potential marker for increased risk of hand eczema in patients with atopic dermatitis, although this hypothesis needs further investigation.¹⁰ A case-control study by de Jongh et al.,¹² performed in 296 patients with chronic irritant contact dermatitis on the hands or forearms, found that patients with FLG null mutations had an increased risk of chronic irritant contact dermatitis. They also hypothesized that besides a history of atopy, information on FLG mutation status could have predictive value for the development of hand eczema.¹² Molin et al.¹³ investigated the association between chronic hand eczema and FLG null mutations in 122 patients (none with atopic dermatitis or ichthyosis).

They found no overall association with hand eczema, but a positive association with combined irritant and allergic contact dermatitis.¹³ Recently, we found a positive but nonsignificant association between hand eczema and FLG null mutations in patients from a tertiary clinic in Denmark.¹⁴ Thus, it remains unclear whether FLG null mutations increase the overall risk of hand eczema or whether these mutations only increase the risk of hand eczema in subjects with atopic dermatitis.

In this study we investigated the possible association between hand eczema and FLG null mutations by using data from a recent general population study performed in Copenhagen, Denmark. A total of 3335 adults were questioned, patch tested and genotyped for the two most common FLG null mutations.

Materials and methods

Study populations

Between June 2006 and May 2008, a cross-sectional study was performed in the general population in Copenhagen. A random sample of 7931 subjects aged 18–69 years was obtained from the Danish Central Personal Register, Ministry of Internal Affairs. All were adults with Danish citizenship and born in Denmark. A total of 3471 (43.7%) subjects participated in a general health examination, 3460 (99.7%) were patch tested and 3346 (96.4%) were FLG genotyped for the 2282del4 and R501X mutations. The participation rate was higher among older age groups.¹⁵ The Ethical Committee of Copenhagen County approved the study (KA-20060011). A written informed consent form was obtained from all participants prior to the beginning of the study.

Questionnaire

All participants were mailed a standard invitation letter and a questionnaire on health, lifestyle and socioeconomic factors. Before the questions on eczema, the questionnaire included a description of eczema: 'Eczema is an itching skin disorder showing redness, dryness, and possibly vesicles and exudation. Eczema is present at the same area for some time'. Participants were asked 'Have you ever had hand eczema?' Participants who gave an affirmative answer were further asked 'Have you had hand eczema within the past 12 months?', 'At what age did you have hand eczema on the first occurrence?' (< 6 years, 6–14 years, 15–18 years, > 18 years), 'How often have you suffered from hand eczema?' [only one time (< 2 weeks), only one time (> 2 weeks), several times, nearly all the time].

A history of atopic dermatitis was defined by the U.K. Working Party's diagnostic criteria for atopic dermatitis as a history of an itchy skin condition plus a minimum of two of four minor criteria.¹⁶ The minor criteria were: (i) a history of involvement of the skin creases; (ii) a personal history of asthma or hay fever; (iii) a history of general dry skin in the last year; and (iv) onset under the age of 2 years.¹⁶

Patch testing

Patch testing was performed by using the panels 1 and 2 of the standardized ready-to-apply TRUE-test (Mekos Laboratories, Hillerød, Denmark).¹⁷ Directions to apply the patch test panels to the upper back 2 days before examination were mailed together with the patch tests. They were read and photographed 1–1.5 h after removal by trained healthcare personnel (supervised by J.P.L. and A.L.). Photos were reviewed by T.M., N.H.N., A.L. and J.P.L. This was done to ensure that the International Contact Dermatitis Research Group (ICDRG) criteria were used consistently.¹⁸ Contact allergy was defined as a positive (at least grade 1+ according to ICDRG) patch test to at least one allergen or mixes of haptens.

FLG genotyping

Regions covering the mutations R501X and 2282del4 of the FLG gene were amplified from genomic DNA by polymerase chain reaction, and the obtained DNA fragments were hybridized to both mutation-specific and wild-type-specific probes attached to paramagnetic beads (Luminex, Austin, TX, U.S.A.). They were subsequently analysed on a BioPlex 200 (Biorad, Hercules, CA, U.S.A.).

Statistical analysis

Deviation from the Hardy–Weinberg equilibrium was tested using the free online calculator at the Online Encyclopedia for Genetic Epidemiology studies (<http://www.oeg.org/software/hwe-mr-calc.shtml>) for both FLG null mutations. We constructed a variable with four different combinations of atopic dermatitis and FLG mutation status: 'group 1' had normal FLG mutation status and absence of atopic dermatitis; 'group 2' had presence of FLG null mutations and absence of atopic dermatitis; 'group 3' had normal FLG mutation status and presence of atopic dermatitis; 'group 4' had presence of FLG null mutations as well as atopic dermatitis. This variable was stratified by hand eczema and contact allergy. The χ^2 trend test (univariate analysis) was used to test for statistically significant differences across the categories of this variable.

A logistic regression model was performed with 'hand eczema within the past 12 months' as the dependent variable, and with sex, age group ('18–35 years', '36–55 years', '56–69 years'), contact allergy ('yes', 'no'), atopic dermatitis ('yes', 'no') and FLG mutation status ('wild-type', 'null mutation') as the independent variables. In this model, tests for interaction between atopic dermatitis and FLG mutation status as well as between sex and FLG mutation status, respectively, were performed by using a log-likelihood ratio test. Another logistic regression analysis was performed with 'hand eczema within the past 12 months' as the dependent variable and with the following independent variables: sex, age group ('18–35 years', '36–55 years', '56–69 years'), contact allergy ('yes', 'no'), and combined FLG mutation and atopic dermatitis status ('normal FLG mutation status and absence of atopic

dermatitis', 'FLG null mutation status and absence of atopic dermatitis', 'normal FLG mutation status and presence of atopic dermatitis', 'FLG null mutation status and presence of atopic dermatitis'.

Associations were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Data analyses were performed using the Statistical Products and Service Solutions package (SPSS Inc., Chicago, IL, U.S.A.) for Windows (release 15.0).

Results

The genotyping success rate was 99.5% for both mutations. A total of 3335 participants (55.3% women and 44.7% men) were patch tested and tested for the R501X and 2282del4 FLG null mutations. The observed genotype prevalences of both polymorphisms did not deviate significantly ($P > 0.05$) from the expected prevalences under the Hardy–Weinberg equilibrium assumption. FLG mutation status among the participants has previously been published¹⁹ and revealed that the combined carrier frequency was 8.1%. The overall prevalence of self-reported hand eczema within the past 12 months and atopic dermatitis was 9.9% (men 8.3% vs. women 11.9%) and 10.1% (men 6.4% vs. women 13.1%), respectively.

We evaluated whether or not the effect of FLG null mutation status on the prevalence of hand eczema was dependent

on sex. Thus, a logistic regression analysis was performed with hand eczema within the past 12 months as the dependent variable and with the following explanatory variables: sex, age group, atopic dermatitis, FLG mutation status and contact allergy. In this model, a test for interaction between gender and FLG mutation status was performed by using a log-likelihood ratio test. The analysis revealed a nonsignificant P -value of the interaction term ($P = 0.22$). The result means that the possible effect of FLG null mutations on the prevalence of hand eczema within the past 12 months did not differ significantly between men and women. Stratification by sex was therefore not performed in our analyses.

We found linear trends of hand eczema, early onset of hand eczema, hand eczema persistence and contact allergy across different groups (groups 1–4) of FLG mutation and atopic dermatitis status (Table 1). Thus, subjects with combined presence of atopic dermatitis and FLG null mutation status had a higher prevalence of hand eczema, an earlier onset and a higher persistence of hand eczema compared with subjects with normal FLG status and absence of atopic dermatitis. Furthermore, the frequency of contact allergy increased across these groups from 9.4% in subjects with normal FLG status and without atopic dermatitis to 17.9% in subjects with presence of FLG null mutations and atopic dermatitis. The increase was not explained by an increase in nickel allergy (Table 1), but by increasing prevalence of contact allergy to ethylenedia-

Table 1 Hand eczema stratified by four combinations of atopic dermatitis and FLG mutation status

	Atopic dermatitis and FLG null mutation status				χ^2 , P-trend
	Group 1 (–atopic dermatitis, –FLG mutation) % (n)	Group 2 (–atopic dermatitis, +FLG mutation) % (n)	Group 3 (+atopic dermatitis, –FLG mutation) % (n)	Group 4 (+atopic dermatitis, +FLG mutation) % (n)	
Hand eczema ever	18.8 (500/2665)	17.7 (35/198)	48.1 (128/266)	62.5 (35/56)	0.001
Hand eczema within past 12 months	44.1 (220/499)	40.0 (14/35)	48.4 (62/128)	71.4 (25/35)	0.02
Age at first onset of hand eczema (years)					
< 6	2.0 (10/501)	5.7 (2/35)	10.9 (14/128)	35.3 (12/34)	0.001
6–14	10.6 (53/501)	8.6 (3/35)	14.1 (18/128)	11.8 (4/34)	
15–18	10.6 (53/501)	17.1 (6/35)	16.4 (21/75)	17.6 (6/34)	
> 18	76.8 (385/501)	68.6 (24/35)	58.6 (75/128)	35.3 (12/34)	
Hand eczema persistence					
One time only (< 2 weeks duration)	26.2 (129/492)	20.6 (7/34)	22.0 (28/127)	15.2 (5/33)	0.03
One time only (> 2 weeks)	11.0 (54/492)	17.6 (6/34)	7.1 (9/127)	3.0 (1/33)	
Several times	55.9 (275/492)	52.9 (18/34)	60.6 (77/127)	51.5 (17/33)	
Nearly all the time	6.9 (34/492)	8.8 (3/34)	10.2 (13/127)	30.3 (10/33)	
Contact allergy					
At least one allergen ^a	9.4 (251/2677)	10.4 (21/201)	12.7 (34/268)	17.9 (10/56)	0.01
Nickel	6.2 (161/2585)	5.5 (11/193)	5.7 (14/253)	7.4 (4/54)	0.94
Fragrance mix I	1.5 (38/2607)	0.5 (1/194)	3.0 (8/263)	5.4 (3/56)	0.02
Ethylenediamine	0.1 (2/2587)	0.5 (1/194)	0.8 (2/258)	4.0 (2/50)	0.001
Neomycin	0 (1/2630)	0 (0/199)	0 (0/263)	1.8 (1/55)	0.01

–Atopic dermatitis, absence of atopic dermatitis; +atopic dermatitis, presence of atopic dermatitis; –FLG mutation, normal FLG status; +FLG mutation, FLG null mutation status. ^aAllergy to at least one of 24 contact allergens from the TRUE test panels 1 and 2.

mine, neomycin and fragrance mix I in subjects belonging to group 4.

We also evaluated whether or not the effect of FLG null mutation status was dependent on atopic dermatitis status. A test for interaction between atopic dermatitis and FLG mutation status was inserted in a logistic regression analysis with hand eczema within the past 12 months as the dependent variable and with the following explanatory variables: sex, age group, atopic dermatitis, FLG mutation status and contact allergy. The analysis revealed a weakly significant P-value of the interaction term ($P = 0.03$). The result means that the effect of FLG null mutations on the prevalence of hand eczema within the past 12 months differed significantly between those with or without atopic dermatitis. Two similar logistic regression analyses were therefore performed with hand eczema within the past 12 months as the dependent variable in 'participants with atopic dermatitis' and in 'participants without atopic dermatitis', respectively (Table 2). We found positive associations between hand eczema within the past 12 months and FLG null mutation status in participants with a history of atopic dermatitis (OR 2.98; 95% CI 1.27–7.01; $P = 0.012$), but not in subjects without atopic dermatitis (OR 0.82; 95% CI 0.41–1.67; $P = 0.60$). Finally, a logistic regression analysis was performed with 'hand eczema within the past 12 months' as the dependent variable and with the following independent variables: sex, age group ('18–35 years', '36–55 years', '56–69 years'), contact allergy ('yes', 'no'), and combined FLG mutation and atopic dermatitis status (Table 3). It showed that

Table 2 Logistic regression analyses with self-reported 'hand eczema within the past 12 months' as the dependent variable and 'FLG mutation status', 'sex', 'age', and 'contact allergy' as the independent variables. Data from participants with and without self-reported atopic dermatitis were included

Explanatory variables	Adjusted OR ^a (95% CI)	
	Participants without atopic dermatitis (n = 2878)	Participants with atopic dermatitis (n = 324)
FLG null mutations		
No	1 (reference)	1 (reference)
Yes	0.82 (0.41–1.67)	2.98 (1.27–7.01)*
Sex		
Male	1 (reference)	1 (reference)
Female	0.99 (0.69–1.42)	0.96 (0.44–2.07)
Age (years)		
18–35	1 (reference)	1 (reference)
36–55	0.61 (0.38–0.99)*	0.60 (0.27–1.34)
56–69	0.61 (0.36–1.02)	0.98 (0.38–2.48)
Contact allergy		
No	1 (reference)	1 (reference)
Yes	1.11 (0.65–1.89)	0.59 (0.24–1.45)

OR, odds ratio; CI, confidence interval. ^aLogistic regression analysis mutually adjusted for shown variables. *Statistically significant.

Table 3 Logistic regression analysis with 'self-reported hand eczema within the past 12 months' as the dependent variable and 'FLG null mutation and atopic dermatitis status', 'sex', 'age' and 'contact allergy' as the independent variables. Data from all participants were included

Explanatory variables	Adjusted OR ^a (95% CI)
	All participants (n = 3335)
FLG null mutation and atopic dermatitis status	
Group 1 (–atopic dermatitis, –FLG mutation)	1 (reference)
Group 2 (–atopic dermatitis, +FLG mutation)	0.83 (0.41–1.68)
Group 3 (+atopic dermatitis, –FLG mutation)	1.16 (0.78–1.72)
Group 4 (+atopic dermatitis, +FLG mutation)	3.23 (1.51–6.91)*
Sex	
Male	1 (reference)
Female	0.99 (0.71–1.36)
Age (years)	
18–35	1 (reference)
36–55	0.62 (0.41–0.93)*
56–69	0.68 (0.43–1.06)
Contact allergy	
No	1 (reference)
Yes	0.94 (0.60–1.48)

OR, odds ratio; CI, confidence interval; –atopic dermatitis, absence of atopic dermatitis; +atopic dermatitis, presence of atopic dermatitis; –FLG mutation, normal FLG status; +FLG mutation, FLG null mutation status. ^aLogistic regression analysis mutually adjusted for shown variables. *Statistically significant; **trend test.

combined presence of atopic dermatitis and FLG null mutation status was significantly and positively associated with hand eczema within the past 12 months. Also, it revealed a positive trend test across FLG mutation and atopic dermatitis status groups ($P = 0.02$).

Discussion

Presence of FLG null mutations increased the risk of self-reported hand eczema within the past 12 months among adult Danes with atopic dermatitis (Table 2). A similar analysis in subjects without atopic dermatitis showed no effect of FLG null mutation status. Furthermore, we found significantly increasing trends of morbidity across four different categories of atopic dermatitis and FLG mutation status (groups 1–4) (Table 1). Thus, 35.3% of participants with presence of FLG null mutations and atopic dermatitis (group 4) reported hand eczema before 6 years of age, in contrast to only 2.0% of participants with normal FLG status and absence of atopic dermatitis (group 1). Similarly, 30.3% of participants belonging to the atopic dermatitis and FLG null mutation group (group 4) reported hand eczema 'nearly all the time', in contrast to only

6.9% of participants with normal FLG status and absence of atopic dermatitis (group 1). These data indicate that FLG null mutation status is associated with early onset of hand eczema as well as persistence of hand eczema.

Participants with FLG null mutations but without atopic dermatitis (group 2) had a lower risk of hand eczema within the past 12 months than participants with atopic dermatitis and normal FLG mutation status (group 3). This difference could be a result of random error as we found linear trends for 'age at onset of hand eczema' and 'hand eczema persistence'. However, they may also underscore that, for example, other hereditary, immunological and/or environmental factors are of stronger relevance in the development of hand eczema than filaggrin deficiency. Thus, the skin barrier dysfunction observed in subjects with FLG null mutations is only one of several aetiological factors of hand eczema. We were not able to adjust for occupational and leisure time exposure to water. A case-control study found that patients with FLG null mutations had an increased risk of chronic irritant contact dermatitis.¹² Molin *et al.*¹³ found no overall association with hand eczema but a positive association with combined irritant and allergic contact dermatitis. Scharschmidt *et al.*²⁰ showed that filaggrin deficiency provoked a paracellular barrier abnormality in mice that reduced inflammatory thresholds to topical irritants and haptens. They hypothesized that it could account for the enhanced antigen penetration in filaggrin-associated atopic dermatitis.²⁰ A large Danish twin study from the general population was not able to find an association between wet work and hand eczema.² The authors speculated that wet work was only a risk factor for a subgroup of people and not for the population as a whole.² Future studies could focus on this issue, as it is possible that FLG mutation status is of particular relevance in those who do extensive wet work. Environmental exposure to water and irritants may have an uneven effect on the risk of hand eczema in subjects with and without a genetically compromised epidermal barrier.

The prevalence of contact allergy to at least one allergen increased significantly across subcategories (groups 1–4) of FLG mutation and atopic dermatitis status (Table 1). Despite low numbers in each cell, we found that the increase was mainly explained by increasing prevalences of allergy to ethylenediamine (a sensitizer used in topical antibiotic and corticosteroid creams), neomycin (an antibacterial that is used widely in topical creams, eye preparations and eardrops) and fragrance mix I. As we have previously discussed, the increase could be explained by more frequent exposure to topical therapy in subjects with a disrupted skin barrier.¹⁹ In Denmark, the product 'Kenalog® Comp. with Mycostatin' (internationally known as Mycolog; Bristol Myers Squibb Pharmaceuticals Ltd, Uxbridge, U.K.) was sold until recently and contained both ethylenediamine and neomycin. The observed increase shows that contact sensitization to specific chemicals is related to treatment exposure. The higher prevalence of fragrance mix I allergy is likely to be explained by fragrances in creams, but could also be a result of increased susceptibility in filaggrin-

deficient subjects. Two recent general population studies have found a positive association between nickel allergy and FLG null mutations, especially in women without ear-piercings.^{19,21} Filaggrin proteins are histidine-rich and may possibly chelate nickel in the epidermis and prevent nickel absorption through skin compartments.²² Thus, ear-piercings may result in direct exposure of nickel ions to immune cells as cutaneous penetration of nickel ions through histidine-rich compartments is bypassed.²² It is currently unknown whether or not the association between hand eczema and nickel allergy is stronger in subjects with FLG null mutation status when compared with subjects with wild-type FLG status. Future studies should address this topic. It has been shown that filaggrin-deficient skin may increase penetration of haptens. One animal study showed that frameshift mutations in the mouse *Flg* gene facilitated allergen penetration (ovalbumin) and development of allergen-specific antibody response following topical application.²³ Furthermore, one case-control study showed that FLG null mutations were positively and significantly associated with allergic sensitization and asthma, suggesting that an impaired skin barrier due to filaggrin deficiency is an important prerequisite for the development subsequent allergic sensitization.²⁴ It is currently unknown if hapten penetration in atopic subjects with filaggrin-deficient skin increases the risk of developing hand eczema. This would also be interesting to determine.

Our study design had strengths and weaknesses that should be briefly addressed. We only performed genotyping for the two most prevalent FLG null mutations, R501X and 2282del4, which cover approximately 85% of null mutations in the FLG gene among caucasians.²⁵ Remaining null mutations in the FLG gene could be unevenly distributed between those with and without hand eczema. This could potentially have introduced bias in our data analyses. Our question on hand eczema within the past 12 months has previously been validated among car mechanics, dentists and office workers.²⁶ Meding and Barregard²⁶ found that the sensitivity was 53–59% and that the specificity was 96–99%. Although it has not been validated in the general population, the levels are likely to be similar. Finally, we estimated the occurrence of atopic dermatitis according to the U.K. Working Party's criteria. Williams *et al.*¹⁶ found that the sensitivity and specificity were 92% and 81%, respectively.

In conclusion, we found that FLG null mutations were significantly associated with hand eczema (< 12 months) in subjects with atopic dermatitis. Furthermore, combined presence of atopic dermatitis and FLG null mutation status was strongly associated with early onset of hand eczema and hand eczema persistence. These results were in accordance with previous studies that have suggested an association between FLG null mutations and hand eczema in atopic individuals.^{10,12,14} Future studies should focus on the possible aggravating effect of wet work and whether the effect of wet work is stronger in FLG null mutation carriers. Also, it is possible that specific hand eczema phenotypes are associated with severe disease.^{14,27}

What's already known about this topic?

- It has been debated in three recent studies whether or not FLG null mutation status is a risk factor for hand eczema.
- The studies have been of limited size and have found associations with chronic irritant hand dermatitis as well as combined irritant and allergic hand eczema.

What does this study add?

- This study shows for the first time that FLG null mutation status increases the risk of hand eczema in atopic individuals.
- Furthermore, it shows an association with onset of hand eczema and disease persistence.

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References

- 1 Thyssen JP, Johansen JD, Linneberg A *et al.* The epidemiology of hand eczema in the general population – prevalence and main findings. *Contact Dermatitis* 2010; **62**:75–87.
- 2 Bryld LE, Hindsberger C, Kyvik KO *et al.* Risk factors influencing the development of hand eczema in a population-based twin sample. *Br J Dermatol* 2003; **149**:1214–20.
- 3 Mortz CG, Lauritsen JM, Bindslev-Jensen C *et al.* Prevalence of atopic dermatitis, asthma, allergic rhinitis, and hand and contact dermatitis in adolescents. The Odense Adolescence Cohort Study on Atopic Diseases and Dermatitis. *Br J Dermatol* 2001; **144**:523–32.
- 4 Dotterud LK, Falk ES. Contact allergy in relation to hand eczema and atopic diseases in north Norwegian schoolchildren. *Acta Paediatr* 1995; **84**:402–6.
- 5 Josefson A, Farm G, Stymne B *et al.* Nickel allergy and hand eczema – a 20-year follow up. *Contact Dermatitis* 2006; **55**:286–90.
- 6 Meding B, Swanbeck G. Predictive factors for hand eczema. *Contact Dermatitis* 1990; **23**:154–61.
- 7 Meding B, Jarvholm B. Hand eczema in Swedish adults – changes in prevalence between 1983 and 1996. *J Invest Dermatol* 2002; **118**:719–23.
- 8 Yngveson M, Svensson A, Johannisson A *et al.* Hand dermatitis in upper secondary school pupils: 2-year comparison and follow-up. *Br J Dermatol* 2000; **142**:485–9.
- 9 Bryld LE, Agner T, Kyvik KO *et al.* Hand eczema in twins: a questionnaire investigation. *Br J Dermatol* 2000; **142**:298–305.
- 10 Lerbaek A, Bisgaard H, Agner T *et al.* Filaggrin null alleles are not associated with hand eczema or contact allergy. *Br J Dermatol* 2007; **157**:1199–204.
- 11 Brown SJ, Cordell HJ. Are filaggrin mutations associated with hand eczema or contact allergy? We do not know. *Br J Dermatol* 2008; **158**:1383–4.
- 12 de Jongh CM, Khrenova L, Verberk MM *et al.* 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**:621–7.
- 13 Molin S, Vollmer S, Weiss EH *et al.* Filaggrin mutations may confer susceptibility to chronic hand eczema characterized by combined allergic and irritant contact dermatitis. *Br J Dermatol* 2009; **161**:801–7.
- 14 Thyssen JP, Carlsen BC, Johansen JD *et al.* Filaggrin haploinsufficiency among patients with dermatitis from a tertiary referral center – early findings and possible phenotype. *Contact Dermatitis* 2010; **62**:182–3.
- 15 Thyssen JP, Linneberg A, Menné T *et al.* The prevalence and morbidity of sensitization to fragrance mix I in the general population. *Br J Dermatol* 2009; **161**:95–101.
- 16 Williams HC, Burney PG, Pembroke AC *et al.* The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994; **131**:406–16.
- 17 Thyssen JP, Linneberg A, Menné T *et al.* Contact allergy to allergens of the TRUE-test (panels 1 and 2) has decreased modestly in the general population. *Br J Dermatol* 2009; **161**:1124–9.
- 18 Wilkinson DS, Fregert S, Magnusson B *et al.* Terminology of contact dermatitis. *Acta Derm Venereol* 1970; **50**:287–92.
- 19 Thyssen JP, Johansen JD, 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; March 25 [Epub ahead of print].
- 20 Schar Schmidt 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.
- 21 Novak N, Baurecht H, Schafer T *et al.* Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. *J Invest Dermatol* 2008; **128**:1430–5.
- 22 Thyssen JP, Carlsen BC, Menné T. Nickel sensitization, hand eczema, and loss-of-function mutations in the filaggrin gene. *Dermatitis* 2008; **19**:303–7.
- 23 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**:602–8.
- 24 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**:214–19.
- 25 O'Regan GM, Sandilands A, McLean WH *et al.* Filaggrin in atopic dermatitis. *J Allergy Clin Immunol* 2008; **122**:689–93.
- 26 Meding B, Barregard L. Validity of self-reports of hand eczema. *Contact Dermatitis* 2001; **45**:99–103.
- 27 Brown SJ, Relton CL, Liao H *et al.* Filaggrin haploinsufficiency is highly penetrant and is associated with increased severity of eczema: further delineation of the skin phenotype in a prospective epidemiological study of 792 school children. *Br J Dermatol* 2009; **161**:884–9.