

Filaggrin mutations are strongly associated with contact sensitization in individuals with dermatitis

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Summary

Background. Although heterozygous filaggrin gene (*FLG*) mutation carriers seem to have an increased risk of atopic, irritant and allergic nickel dermatitis, it remains unclear whether the risk of contact sensitization to allergens other than nickel is also elevated in *FLG* mutation carriers.

Objectives. We hypothesized that heterozygous *FLG* mutation carriers who suffer from dermatitis will have strongly reduced or even absent filaggrin levels during episodes of inflammation, potentially increasing the penetration of contact allergens, and hence the risk of becoming sensitized.

Materials and methods. During 2006–2008, 3335 randomly invited 18–69-year-old adult Danes participated in a general health examination, filled out a questionnaire, and were genotyped for the R501X and 2282del4 mutations in *FLG*.

Results. A logistic regression analysis restricted to individuals who reported atopic dermatitis and frequent episodes of hand eczema showed a strong association between *FLG* mutations and contact sensitization to allergens other than nickel (odds ratio 5.71; 95% confidence interval 1.31–24.94). In participants without dermatitis, no association was found between contact sensitization and *FLG* mutations.

Conclusion. *FLG* mutation carriers with self-reported dermatitis have an increased risk of contact sensitization to substances other than nickel, whereas *FLG* mutations alone may not, or may only slightly, increase the risk of sensitization.

Key words: contact sensitization; dermatitis; epidemiology; filaggrin gene; general population.

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Filaggrin proteins are crucial for skin barrier functions, as they guarantee the alignment of keratin filaments in the corneocytes and hydrate the stratum corneum. Successful genotyping of the filaggrin gene (*FLG*) showed that loss-of-function mutations were not only very prevalent in European and Asian races, but also caused xerosis and increased the risk of dermatitis, owing to lower filaggrin and filaggrin metabolite levels (1, 2). Moreover, T helper cell type 2-driven immune responses, characterized by, for example, elevated interleukin (IL)-4, IL-13, IL-17A, IL-22 and IL-25 levels, may reduce filaggrin levels, and cause secondary impairment of the skin barrier (3–6).

Table 1. The prevalence of dermatitis and contact sensitization stratified by filaggrin gene (*FLG*) mutation status

	<i>FLG</i> mutation status			Crude odds ratio (95% confidence interval)
	All, % (no.)	Null, % (no.)	Wild type, % (no.)	
Contact sensitization (not to nickel and thimerosal)	4.0 (132/3335)	6.3 (17/269)	3.8 (115/3066)	1.73 (1.03–2.93)
Atopic dermatitis	10.1 (324/3202)	21.8 (56/257)	9.1 (268/2945)	2.78 (2.02–3.84)
Hand eczema ever	21.7 (716/3295)	26.9 (71/264)	21.3 (645/3031)	1.36 (1.02–1.81)
Only one episode of hand eczema	7.6 (249/3282)	7.3 (19/261)	7.6 (230/3021)	0.96 (0.59–1.57)
Frequent episodes of hand eczema	13.6 (454/3282)	18.8 (49/261)	13.4 (405/3021)	1.39 (1.01–1.92)
Atopic dermatitis + hand eczema ever	4.8 (159/3312)	7.9 (21/267)	4.5 (138/3045)	1.80 (1.12–2.90)
Atopic dermatitis + frequent episodes of hand eczema	3.6 (117/3219)	10.5 (27/258)	3.0 (90/2961)	3.72 (2.38–5.85)

Null = Presence of at least a single mutation in *FLG*; R501X or 2282del4.

Heterozygous *FLG* mutation carriers seem to have an increased risk of nickel sensitization and a lower age at onset of allergic nickel dermatitis (7–9), presumably owing to reduced levels of nickel-chelating histidine residues, which may facilitate penetration of nickel ions through the stratum corneum. However, it remains unclear whether the risk of contact sensitization to allergens other than nickel is also elevated in mutation carriers. Hence, no differences in prevalence rates of contact sensitization (not nickel) between *FLG* mutation carriers and non-carriers were found in large cohorts of adult Germans and Danes from the general population (7, 9). On the other hand, filaggrin-deficient mice had reduced inflammatory threshold levels for the contact allergen oxazolone than wild-type mice (10), and the prevalence of contact sensitization to allergens found in topical products such as neomycin, ethylenediamine and fragrances was slightly higher in *FLG* mutation carriers with atopic dermatitis than in controls (11). However, it should be emphasized that, in the case of exposure to potent allergens and/or high concentrations of the allergens, the effect of genetic predisposition to contact sensitization will be overruled (12). Here, we re-investigated the association between *FLG* mutations and contact sensitization. We focused on individuals who reported atopic dermatitis or hand eczema, as we hypothesized that heterozygous *FLG* mutation carriers who suffer from dermatitis will have strongly reduced or even absent filaggrin levels during episodes of inflammation, together with increased immune cell levels, potentially increasing the penetration of contact allergens and hence the risk of becoming sensitized.

Table 2. Results of logistic regression analyses with 'contact sensitization (but not to nickel and thimerosal)' as the dependent variable and filaggrin gene (*FLG*) mutation status, sex, age and atopic dermatitis as the explanatory variables in different subgroups

Analysis restricted to subgroup	Adjusted odds ratio of being <i>FLG</i> mutation carrier with 95% confidence interval*
All	1.57 (0.92–2.69)
Never dermatitis (atopic, or hand eczema)	1.22 (0.52–2.83)
Atopic dermatitis	2.08 (0.86–5.04)
Hand eczema ever	2.46 (1.09–5.55)
Only one episode of hand eczema	0.99 (0.11–8.73)
Frequent episodes of hand eczema	4.57 (1.75–11.93)
Atopic dermatitis + hand eczema ever	3.45 (1.07–11.08)
Atopic dermatitis + frequent episodes of hand eczema	5.71 (1.31–24.94)

*Besides atopic dermatitis and *FLG* mutations, adjustment was performed for sex and age.

Materials and Methods

During 2006–2008, 3471 (43.7%) of 7931 randomly invited 18–69-year-old adult Danes born in Denmark participated in a general health examination, and 3335 (96.1%) were genotyped for the R501X and 2282del4 mutations in *FLG* (9, 13, 14). Patch testing was performed by using panels 1 and 2 of the standardized ready to apply TRUE Test® (Mekos Laboratories, Hillerød, Denmark). Contact sensitization was defined as a positive (at least grade 1+ according to the International Contact Dermatitis Research Group criteria) patch test reaction to at least one allergen or mixes of haptens on day 2, but not to nickel and/or thimerosal. The latter allergens were excluded because sensitization is often related to

piercing and vaccinations, events in which the stratum corneum is bypassed, potentially introducing a bias (14). Participants were asked about general health, lifestyle and socio-economic factors. Questions included, 'have you ever had hand eczema?', and 'if so, how often?' ('only once', 'several times or all the time'). Atopic dermatitis was defined by the UK Working Party's diagnostic criteria as a history of an itchy skin condition within the past year plus a minimum of two of four minor criteria (15). No clinical examination for atopic dermatitis was performed. Characteristics of participants were compared by use of the chi-square (χ^2) test. Logistic regression models were used for association testing. Data analyses were performed with the SPSS package (SPSS™, Chicago, IL, USA) for Windows™ (release 15.0). Odds ratio (ORs) and 95% confidence intervals (CIs) are given.

Results

The prevalence of the combined *FLG* mutations was 8.0% (269/3335); there were 0.2% ($n = 9$) homozygous and 7.8% ($n = 260$) heterozygous carriers. Contact sensitization to at least one allergen (but not nickel and thimerosal), atopic dermatitis and hand eczema were stratified by *FLG* mutation status (Table 1). An adjusted regression analysis with contact sensitization as the dependent variable and age, sex, atopic dermatitis and *FLG* mutations as the explanatory variables showed that both heterozygous and homozygous carrier status was positively, but non-significantly, associated with contact sensitization [the ORs with 95% CIs were, respectively, 1.56 (0.89–2.70) and 1.84 (0.22–15.44)]. In similar regression analyses restricted to individuals who reported atopic dermatitis and/or hand eczema, but with combined heterozygous and homozygous mutation status, strong and significant associations were found between *FLG* mutations and contact sensitization (Table 2). Finally, in all participants, a logistic regression analysis was performed with contact sensitization as the dependent variable and with the following explanatory variables: sex, age, and a categorical variable where the reference value was defined by the 'absence of *FLG* mutations', and the two values 'presence of *FLG* mutations but absence of dermatitis' and 'presence of *FLG* mutations and dermatitis' were compared with the reference value. Here, the ORs for

the last two variables with 95% CIs were, respectively, 0.99 (0.43–2.28) and 3.37 (1.74–6.53). No association with nickel sensitization was found in individuals with and without dermatitis; however, in a previous study we showed a positive association in women without ear piercing (9).

Discussion

This study suggests that *FLG* mutation carriers with self-reported dermatitis have an increased risk of contact sensitization, whereas *FLG* mutations alone may not, or may only slightly, increase the risk. By using cross-sectional data, we could not determine whether contact sensitization preceded dermatitis or vice versa. However, as many allergens are also irritants causing cytokine release and recruiting immune cells, concomitant irritant and allergic contact dermatitis is probably a relatively common phenomenon. We suggest that, during episodes of primary dermatitis, whether irritant or atopic in nature, an inflammatory driven downregulation of filaggrin expression is likely to result in very low or even absent levels of filaggrin proteins in the skin. This will further decrease skin barrier function and increase contact allergen penetration when the skin is exposed to such allergens, ultimately inducing secondary contact sensitization. Although, clearly, the present study design cannot confirm this hypothesis, experimental investigations should be conducted to challenge it. Hence, we highlight the possibility that the association could also be explained by primary contact sensitization causing allergic contact dermatitis. Individuals with frequent episodes of hand eczema had a higher risk of contact sensitization, because they may have had more exposure to both irritants and allergens than, for example, children with atopic dermatitis. A small case–control study showing a positive association between *FLG* mutations and combined irritant and allergic contact dermatitis supports our data to some degree (16). Healthcare providers who see patients with dermatitis may benefit from knowing their *FLG* genotype, as these patients should be treated with low-allergenicity topical products, and be particularly advised against exposure to contact allergens during episodes of dermatitis.

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