

Carriers of filaggrin gene (*FLG*) mutations avoid professional exposure to irritants in adulthood

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Summary

Background. Loss-of-function mutations in the filaggrin gene (*FLG*) are associated with xerosis, atopic dermatitis, and early onset of hand eczema. Irritant exposure is a risk factor for occupational hand eczema, and *FLG* mutations increase the risk of occupational irritant contact dermatitis on the hands in hospital cohorts. It is unknown whether *FLG* mutations affect the level of irritant exposure.

Objectives. To evaluate whether exposure to occupational irritants was dependent on *FLG* mutations, atopic dermatitis, and age at hand eczema onset.

Methods. Randomly chosen Danish adults completed a questionnaire on general health and occupational exposures. Genotyping for *FLG* mutations (R501X, 2282del4, and R2447X) and patch testing were performed.

Results. Overall, 38.7% of subjects reported present or previous occupational exposure to irritants. Among individuals who reported hand eczema onset before entering their work life, 50.6% (45/89) of *FLG* non-mutation carriers became exposed to irritants, as compared with 28.6% (4/14) of heterozygous and 0% (0/6) of homozygous mutation carriers ($p=0.006$). Avoidance was conspicuous among mutation carriers reporting childhood hand eczema and atopic dermatitis (odds ratio 0.08, 95% confidence interval 0.01–0.65).

Conclusions. Carriers of *FLG* mutations who have had hand eczema onset in childhood avoid occupational exposure to irritants; the association is most marked with homozygous mutation status combined with atopic dermatitis.

Key words: filaggrin mutations; hand eczema; occupational irritant exposure.

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Filaggrin proteins help to align keratin filaments in the stratum corneum (1, 2), and are then degraded to hygroscopic amino acids, 'the natural moisturizing factors' (3, 4). When the skin is inflamed, or when ambient humidity drops below 80%, degradation of filaggrin proteins is accelerated to maintain skin hydration (5). Loss-of-function mutations in the filaggrin gene (*FLG*) are observed in ~10% of Caucasian individuals (6), and cause xerosis, scaly skin, and palmar hyperlinearity (7, 8). Pertinently, *FLG* mutations increase the skin pH, reduce hydration, and result in increased transepidermal water loss.

FLG mutations are associated with atopic dermatitis, and early onset and persistence of hand eczema,

with a predilection for the dorsal aspects of the hands (9, 10). An association between *FLG* mutations and irritant contact dermatitis, as well as combined irritant and allergic contact dermatitis on the hands, has been shown in dermatitis patients (11, 12). Additionally, irritant contact dermatitis caused more sick-leave and a three-fold increased risk of job loss in patients with both *FLG* mutations and atopic dermatitis from a tertiary clinic as compared with controls (13), and was independently associated with *FLG* mutations (14). The above findings indicate that *FLG* mutation carriers have particularly sensitive skin, which increases the risk of hand eczema in early childhood, and which, in occupational patients from tertiary hospitals, increases the risk of irritant contact dermatitis and leads to a poor prognosis. Moreover, *FLG* mutations are associated with skin fissures on the hands and fingers, emphasizing the reduced skin hydration (15).

Whereas atopic dermatitis is the single most important risk factor for hand eczema, exposure to irritants is the most common cause of occupational hand eczema (16). Exposure to wet work alone was reported by 20% of employees in Sweden in 2009 (17). Hairdressing is associated with intense exposure to allergens and irritants, and it is therefore noteworthy that the prevalence of atopic dermatitis in hairdressing apprentices was significantly lower than in controls (18). This 'healthy-worker effect' could be explained by healthcare provider advice prior to choice of profession or reluctance to pursue a professional career with irritant exposure in individuals who already suffer from skin barrier problems. This observation led us to hypothesize that there exists a hitherto unrecognized subtype of atopic dermatitis combined with *FLG* loss-of-function mutations where particularly sensitive skin and early-onset hand eczema could affect career choice, directing people towards professions with little or no exposure to irritants.

In this study, using cross-sectional general population data, we investigated whether exposure to occupational irritants was dependent on common *FLG* mutations, atopic dermatitis, and early or late onset of hand eczema.

Materials and Methods

Study population

In 2006–2008, a cross-sectional study was performed in the western part of Copenhagen. A general health examination was offered to 7931 randomly selected individuals aged 18–69 years, and 3471 people participated (43.7%). The participants completed a questionnaire on general health and occupational exposures. The characteristics of the population have been described previously (19).

In total, 3460 (99.7%) individuals were patch tested, and 3346 (96.4%) were genotyped for the three most common Caucasian *FLG* mutations: R501X, 2282del4, and R2447X. The study was approved by the Ethics Committee of Copenhagen County (KA-20060011), and a written informed consent form was obtained from all of the participants.

Questionnaire

Questions concerning the skin were preceded by a description of eczema: 'Eczema is an itching skin disorder showing redness, dryness and possibly vesicles and exudation. Eczema is present in the same area for some time.' Participants who gave an affirmative answer to the question: 'Have you ever had hand eczema' (yes/no), were also asked 'At what age did you have hand eczema on the first occurrence? (before 6 years of age/age 6–14 years/age 15–18 years/after 18 years of age)'. Occupational exposures were assessed with the question: 'Have you, at your past or present workplace, been exposed to any of the following exposures several times a week' (yes/no). Affirmative answers to conditions concerning wet work (partially defined according to the German Approved Code of Practice no. 401) were included in the analyses as occupational irritant exposures: (i) wet work; (ii) use of protective gloves for more than 2 hr/day; and (iii) hand washing at least 20 times daily (20). Additionally, the following exposures were included: 'Cooking or food handling', 'solvents', 'cleaning agents', 'water vapour or moist environments', and 'wet work' (Table 1). The participants were asked to report their educational level based on the categories: 'Skilled or unskilled blue-collar worker', 'short-cycle higher education (< 3 years)', 'medium higher education (3–4 years)', 'long-cycle higher education (> 4 years)', or 'other education'. Atopic dermatitis was defined according to the UK Working Party's diagnostic criteria, with one major criterion and at least two of four minor criteria (21).

Patch testing

Patch testing was performed with the standardized TRUE test[®] (Mekos Laboratories, Hilleroed, Denmark). Readings were performed on D2 only (22). Patch test readings of +1, +2 or +3 were considered to be positive. If a patch did not have direct skin contact upon reading, or if participants had removed individual patches from the test series before application because of known allergy status, data were registered as missing.

Table 1. Self-reported occupational irritant exposures

Exposure type	All, % (n) (n _{total} = 3038)	FLG mutation carriers*, % (n) (n _{total} = 270)	FLG non-mutation carriers*, % (n) (n _{total} = 2768)	χ^2 statistics (p)
Cooking or food handling	11.5 (349)	12.7 (34)	11.4 (315)	0.540
Solvents	10.5 (320)	11.5 (31)	10.5 (289)	0.613
Cleaning agents	14.9 (454)	14.6 (39)	15.1 (415)	0.836
Water vapor or moist environment	6.9 (210)	10.1 (27)	6.7 (183)	0.034
Wet work (unspecified)	7.0 (213)	7.2 (19)	7.1 (194)	0.950
Glove use for at least 2 hr/day	16.4 (499)	19.4 (52)	16.3 (447)	0.193
Hand washing at least 20 times/day	15.4 (467)	15.4 (41)	15.5 (426)	0.966
At least one irritant exposure	38.7 (1176)	38.9 (105)	38.7 (1071)	0.949

*Genotyped for the three most common filaggrin gene (*FLG*) loss-of-function mutations (R501X, 2282del4, and R2447X).

FLG genotyping

Genomic DNA was purified from blood samples, and typed for the *FLG* loss-of-function mutations R501X, 2282del4 and R2447X by means of asymmetric, allele-specific polymerase chain reaction (PCR) and tag-specific microbead detection (23). Briefly, multiplex PCR was performed with an excess of biotinylated reverse primers and tagged forward primers, to create single-stranded, allele-specific PCR products. The PCR products were then hybridized to MagPlex C beads with allele-specific capture probes (Luminex, Austin, TX, USA). Analyses were performed on a Bio-Plex 200 device (Bio-Rad, Hercules, CA, USA).

Statistical analyses

Data were processed in SPSS (SPSS Statistics Inc., Chicago, IL, USA; IBM PASW statistics) for Windows™ (release 15.0 and 18.0). Hardy–Weinberg tests were performed with the Hardy–Weinberg calculator from ‘Online Encyclopedia for Genetic Epidemiology Studies’ (24). The χ^2 -test was used to compare categorical characteristics in different groups. For participants reporting hand eczema before 15 years of age, a logistic regression analysis was performed with occupational irritant exposure (yes/no) as the dependent variable and with *FLG* genotype (mutations/no mutations) combined with atopic dermatitis history (atopic dermatitis/no atopic dermatitis), giving four categories, as the independent variable. In participants who denied having hand eczema onset before 15 years of age, a logistic regression analysis was performed with hand eczema onset after 15 years of age as the dependent variable. The independent variables were: sex (male/female), age (18–35/36–55/56–69 years), educational level (skilled or unskilled blue-collar workers/short-cycle higher education/medium-cycle higher education/long-cycle higher education/other), atopic dermatitis (yes/no),

contact allergy (yes/no), occupational irritant exposure (yes/no), and *FLG* mutations (yes/no). Associations were expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

Results

In total, 3348 individuals were genotyped for the *FLG* loss-of-function mutations R501X, 2282del4, and R2447X. The combined carrier frequency, including both heterozygous and homozygous carriers, was 9.0% (302/3348), and 12 (0.4%) were homozygotes. The population distribution of the 2282del4 mutation was in Hardy–Weinberg equilibrium ($p=0.149$), whereas Hardy–Weinberg testing gave p -values of 0.05 and 0.0007 for R501X and R2447X, respectively.

All participants

Similar proportions of *FLG* mutation carriers and non-mutation carriers reported exposure to at least one type of irritant in their past or present occupation (38.9% versus 38.7%). The homozygous *FLG* mutation carriers reported less occupational irritant exposure than heterozygous and non-mutation carriers, but the difference was not statistically significant [18.2% (2/11), 39.8% (103/259), and 38.7% (1071/2768), respectively, $p=0.354$]. In the case of atopic dermatitis, occupational irritant exposure was reported more often in individuals who had atopic dermatitis than in individuals who did not [45.5% (136/299) versus 37.8% (1036/2739), $p=0.01$]. No difference in the overall number of occupational irritant exposures was observed between mutation carriers and non-mutation carriers (Fig. 1a).

Participants who reported hand eczema at some point

The irritant exposure load was also evaluated among individuals who reported having had hand eczema at

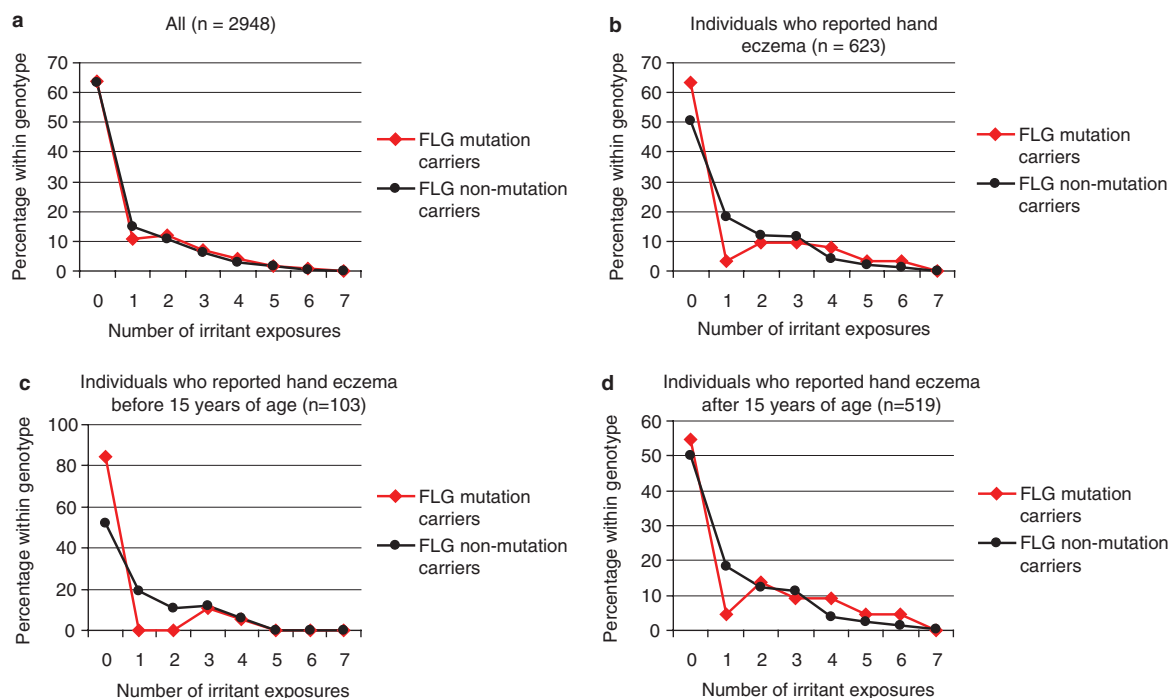


Fig. 1. Number of occupational irritant exposures per individual stratified by filaggrin genotype (*FLG*).

some point in their lives (Fig. 1b). More *FLG* mutation carriers than non-mutation carriers reported absence of occupational irritant exposure [63.5% (40/63) versus 50.5% (283/560), $p = 0.051$], and of the 8 homozygous carriers, none reported occupational irritant exposures. Fewer individuals with *FLG* mutations reported a single irritant exposure [3.2% (2/63) versus 18.4% (103/560), $p = 0.002$] (Fig. 1b)].

Participants who reported having had hand eczema before 15 years of age

We evaluated the irritant exposure pattern among individuals who had their first occurrence of hand eczema before entering their work life and thus exposure to occupational irritants, that is, before 15 years of age in Denmark (Fig. 1c and Table 2). Here, the number of irritant exposures in adulthood differed markedly between *FLG* mutation carriers and non-mutation carriers (Fig. 1c), and avoidance of occupational exposure was associated with *FLG* mutation status in a dose-dependent manner (Table 2). Hence, among individuals who reported childhood hand eczema, 50.6% (45/89) of non-mutation carriers became occupationally exposed to irritants, as compared with 28.6% (4/14) of heterozygous carriers and 0% (0/6) homozygous carriers ($p_{\text{linear-by-linear}} = 0.006$). The four *FLG* mutation

carriers with occupational irritant exposure all reported glove use for more than 2 hr/day, whereas only 37.8% (17/45) of the non-mutation carriers did so ($p = 0.028$). To evaluate whether the observation was confounded by a varying prevalence of atopic dermatitis according to genotype status, we analysed the combined effect of *FLG* mutations and atopic dermatitis (Table 3). Although significantly more *FLG* mutation carriers [70.0% (14/20)] than non-mutation carriers [(30.6% (26/85))] ($p = 0.001$) had atopic dermatitis, avoidance of occupational irritant exposure was not explained by having a history of atopic dermatitis. Hence, non-mutation carriers with atopic dermatitis chose occupations with irritant exposure as frequently as individuals without atopic dermatitis, irrespective of *FLG* genotype status ($\sim 50.0\%$, Table 3). Avoidance of irritant exposure in adulthood was associated with combined *FLG* mutation and atopic dermatitis status ($\text{OR}_{\text{FLG mutations, atopic dermatitis}} = 0.08$, 95% CI 0.01–0.65; Table 3), and 6 of the 6 carriers who were homozygous for *FLG* mutations were found in this group.

Participants who reported hand eczema after 15 years of age

In individuals who reported hand eczema onset after 15 years of age, absence of occupational irritant

Table 2. The implications of filaggrin genotype for occupational irritant exposure in adulthood among individuals who reported hand eczema onset before 15 years of age

		Occupational irritant exposure, % (n/n _{total})	χ^2 statistics
FLG mutation	None	50.6 (45/89)	$p_{\text{linear-by-linear}} = 0.006$
carrier	Heterozygous	28.6 (4/14)	
status*	Homozygous	0.0 (0/6)	
Atopic dermatitis†	Yes	35 (14/40)	$p = 0.197$
	No	47.8 (32/67)	

FLG, fillagrin gene.

*Genotyped for the three most common loss-of-function mutations (R501X, 2282del4, and R2447X).

†Defined according to the UK Working Party's diagnostic criteria for atopic dermatitis (one major criterion and at least two of four minor criteria).

exposure was independent of *FLG* mutation status (Fig. 1d), whereas a tendency not to be exposed to just one occupational irritant was again found among mutation carriers as compared with non-mutation carriers.

A risk factor analysis on hand eczema onset in adulthood (after 15 years of age) is presented in Table 4, and shows that atopic dermatitis ($OR_{\text{crude}} = 3.59$, 95% CI 2.77–4.66; $OR_{\text{adjusted}} = 3.35$, 95% CI 2.48–4.54), contact allergy ($OR_{\text{crude}} = 1.57$, 95% CI 1.19–2.06; $OR_{\text{adjusted}} = 1.39$, 95% CI 1.01–1.91) and occupational

irritant exposure ($OR_{\text{crude}} = 2.04$, 95% CI 1.69–2.46; $OR_{\text{adjusted}} = 1.89$, 95% CI 1.53–2.34) were significantly associated factors, whereas *FLG* mutations did not increase the risk of hand eczema with an adult onset. In the adjusted analysis, the variables sex, age and educational level were included, in addition to the above-mentioned variables.

Discussion

This cross-sectional general population study showed that individuals with and without *FLG* loss-of-function mutations were equally exposed to irritants in their professional lives. However, having *FLG* mutations and hand eczema before the age of 15 years resulted in significantly and markedly reduced occupational exposure to irritants, particularly among the carriers who were homozygous for *FLG* mutations. From the pioneering studies by Rystedt (25), it is clear that individuals with atopic dermatitis and hand eczema in childhood have a particularly high risk of having hand eczema as adults. As we expected that having childhood hand eczema would affect career choice in adulthood, it was decided to perform data analyses taking the age at onset of hand eczema into consideration. This led to the novel finding that only 20% of individuals with childhood hand eczema carrying *FLG* null mutations were occupationally exposed to irritants, as compared with 50% of the non-mutation carriers. Thus, a large group in the population tends to avoid professional exposure to irritants, which can be identified by combining a clinical disease (hand eczema in childhood) and a genotype (*FLG* loss-of-function mutations). Furthermore, it appeared that *FLG* mutation carriers protected their hands by more extensive use of gloves, although large case series are required to confirm this. We also showed that individuals with atopic dermatitis, childhood hand eczema and *FLG* loss-of-function mutations seemed to completely avoid occupational exposure to irritants.

In a recent study on hairdressing apprentices, a significantly lower frequency of atopic dermatitis was found among the apprentices (21.4%) than in a general population control group (29.8%) (18). Earlier studies have shown that there is a group of individuals with atopic dermatitis dominated by females, who develop early-onset occupational hand eczema (26, 27). The lower prevalence of atopic dermatitis in hairdressing apprentices could be explained by successful primary prevention of hand eczema following healthcare provider advice, or by individual avoidance behaviour because of dermatitis. Two recent studies from a German/Dutch group showed that *FLG* mutations alone, and especially

Table 3. Logistic regression analysis on the implications of filaggrin genotype and atopic dermatitis for occupational irritant exposure in adulthood among individuals who reported hand eczema onset before 15 years of age

	Occupational irritant exposure, % (n/n _{total})	OR (95% CI)
No <i>FLG</i> mutations†		
No atopic dermatitis†	49.2 (29/59)	1.00 (reference)
No <i>FLG</i> mutations†		
Atopic dermatitis†	50.0 (13/26)	1.03 (0.41–2.60)
<i>FLG</i> mutations†		
No atopic dermatitis†	50.0 (3/6)	1.03 (0.19–5.55)
<i>FLG</i> mutations†		
Atopic dermatitis†	7.1 (1/14)	0.08 (0.01–0.65)*

CI, confidence interval; *FLG*, fillagrin gene; OR, odds ratio.

* $p < 0.05$.

†At least one of the three most common loss-of-function mutations (R501X, 2282del4, and R2447X).

‡Defined according to the UK Working Party's diagnostic criteria for atopic dermatitis (one major criterion and at least two of four minor criteria).

Table 4. Risk analyses of hand eczema onset in adulthood

		All except individuals who reported hand eczema onset before 15 years of age, % (n/n _{total})	Hand eczema onset in adulthood [†] , % (n/n _{total})	OR for the explanatory variables (95% CI)	
				Crude OR [‡]	Adjusted OR [§]
Atopic dermatitis [¶]	No	91.1 (2807/3082)	16.7 (468/2807)	1.00 (reference)	1.00 (reference)
	Yes	8.9 (275/3082)	41.8 (115/275)	3.59 (2.77–4.66)**	3.35 (2.48–4.54)**
Contact allergy ^{††}	No	90.3 (2867/3173)	18.0 (515/2867)	1.00 (reference)	1.00 (reference)
	Yes	9.7 (309/3176)	25.6 (79/309)	1.57 (1.19–2.06)*	1.39 (1.01–1.91)*
Occupational irritant exposure ^{††}	No	61.6 (1791/2907)	14.3 (256/1791)	1.00 (reference)	1.00 (reference)
	Yes	38.4 (1116/2907)	25.4 (283/1116)	2.04 (1.69–2.46)**	1.89 (1.53–2.34)**
<i>FLG</i> mutations ^{§§}	No	91.4 (2913/3187)	18.6 (543/2913)	1.00 (reference)	1.00 (reference)
	Yes	8.6 (274/3187)	19.0 (52/274)	1.02 (0.75–1.40)	0.95 (0.65–1.38)

CI, confidence interval; *FLR*, fillagrin gene; OR, odds ratio.

*Significant difference in χ^2 -tests, $p < 0.001$.

**Difference in χ^2 -tests, $p < 0.05$.

[†]An affirmative answer to the question: 'Have you ever had hand eczema?' and reporting year of onset ≥ 15 years of age.

[‡]Effect of the noted variable on the risk of hand eczema onset in adulthood (each separately).

[§]Effect of the noted variable on the risk of hand eczema onset in adulthood in a regression analysis including adjustment for sex, age, and educational level.

[¶]Defined according to the UK Working Party's diagnostic criteria for atopic dermatitis (one major criterion and at least two of four minor criteria).

^{††}Defined as positive patch test readings (+, ++, +++) according to the International Contact Dermatitis Research Group criteria.

^{††}An affirmative answer regarding past or present occupational exposure to at least one of the following: cooking or food handling, solvents, cleaning agents, water vapour or moist environment, wet work (unspecified), glove use for at least 2 hr per day, and hand washing at least 20 times per day.

^{§§}At least one of the three most common *FLG* loss-of-function mutations (R501X, 2282del4, and R2447X).

in combination with atopic dermatitis, were associated with occupational irritant contact dermatitis on the hands (13, 14). Furthermore, *FLG* mutations and atopy resulted in a poor prognosis (13). Interestingly, we showed that *FLG* mutations did not constitute an independent risk factor for adult-onset hand eczema in a general population cohort. This finding emphasizes that having an inherited barrier abnormality is of less importance than excessive environmental exposure to irritants and contact allergens for the development of hand eczema. Also, atopic dermatitis was, as expected, a strong risk factor for hand eczema, which could be explained by the chronic capacity in an individual to develop skin inflammation when exposed to various stimuli rather than the barrier abnormality. Our findings fit with the recent findings from Germany and The Netherlands, as *FLG* mutations impair the prognosis markedly once hand eczema has developed because of the barrier abnormality.

Among Danish dermatitis patients with occupational hand eczema, irritant contact dermatitis preceded allergic contact dermatitis, as it occurred at a lower age (27). This finding is in line with immunological and epidemiological data, which were recently reviewed (28). Also, a recent general population study showed that

FLG mutations were strongly associated with contact sensitization in individuals who reported dermatitis (29). One may therefore suggest that the risk of allergic contact dermatitis can ultimately be reduced by limiting the onset and severity of irritant contact dermatitis, especially in those with *FLG* mutations. Future studies are required to investigate this.

The definitions of hand eczema, occupational irritant exposure and atopic dermatitis relied on questionnaire data, which potentially introduce bias and misclassification. However, the questions concerning atopic dermatitis have been validated by the UK Working Party, and have a high specificity and a moderate sensitivity (21). Unfortunately, we did not have detailed information about irritant exposure. Patch test readings were performed only on D2, which may cause both false-negative and false-positive reactions. Nonetheless, studies like this usually convey important information on the burden of disease in a general population. Owing to a lack of the relevant parameters, we were not able to evaluate the course of hand eczema in individuals who reported childhood hand eczema and yet became occupationally exposed to irritants.

In summary, *FLG* mutations combined with childhood hand eczema onset result in reduced occupational

exposure to irritants, which is most markedly associated with homozygote status combined with atopic dermatitis. On the basis of the recent finding that *FLG* mutation carriers have an increased risk of occupational irritant contact dermatitis and a poor prognosis (13, 14), health-care providers should continue to take specific preventive measures for this particular group of individuals.

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