

Nickel reactivity and filaggrin null mutations – evaluation of the filaggrin bypass theory in a general population

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Summary

Background. It was recently shown that filaggrin null mutation carrier status was associated with nickel allergy and self-reported intolerance to costume jewellery. Because of the biochemical characteristics of filaggrin, it may show nickel barrier properties in the stratum corneum.

Objectives. To investigate whether subjects with filaggrin null mutations report nickel dermatitis at an earlier age than wild-type individuals, and to analyse whether null mutation carriers have stronger patch test reactivity to nickel sulfate than do wild-type individuals.

Materials. A total of 3471 Danes (18–69 years of age) answered a questionnaire about general health, and underwent patch testing and filaggrin genotyping.

Results. The mean number of years at risk of developing nickel dermatitis was significantly lower for the filaggrin null genotype than for the wild-type genotype when ear piercing status was considered. In positive patch test readings, the proportion of null mutants increased with increasing reaction strength.

Conclusions. Filaggrin null mutations may lower the age of onset of nickel dermatitis. The hypothesis that ear piercings obscure the effect of filaggrin null mutations on the development of nickel allergy in statistical analyses was supported. An association between the null genotype and increased nickel sensitivity was indicated by patch test reading and questionnaire data.

Key words: allergy, dermatitis, filaggrin, nickel, null mutations, patch test reactivity.

Filaggrin (FLG) is an epidermal protein found in the stratum corneum, where it has a structural as well as a moisturizing function (1). *FLG* null mutation homozygosity is rare, whereas approximately 8% of adult Danes are heterozygous carriers of the two most frequent Caucasian

null mutations (2, 3). Novak et al. recently showed that *FLG* null mutation carrier status was associated with nickel allergy and self-reported intolerance to costume jewellery (4).

We have suggested that *FLG* null mutation status may increase nickel penetration through the epidermis (5). As skin is a complex biological matrix, penetration of metal ions is determined by a variety of factors (6). It is known that nickel accumulates in the stratum corneum (7) and, on the basis of binding studies, Fullerton et al. hypothesized that a gradual build-up of local nickel reservoirs could lead to saturation and ultimately free passage of nickel ions through the skin (8). Because of the biochemical characteristics of FLG, which is relatively

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histidine-rich (9), and the fact that histidine strongly chelates nickel, the presence of filaggrin may be important for the nickel-binding capacity of the stratum corneum. Thus, FLG proteins may prevent free nickel ion passage to deeper skin compartments, where interaction with the immune system can provoke nickel sensitization and elicit an allergic response (5). According to the recently proposed bypass theory, a piercing event (with a nickel-releasing piercing post) would bypass the skin barrier effect of filaggrin proteins, as the barrier is perforated (5). Thus, the epidemiological effect of *FLG* null mutations is difficult to study in pierced individuals (2).

Given that nickel and, perhaps, other metal ions are held in the stratum corneum by direct interaction with FLG, its derivatives and, probably, other proteins, *FLG* null mutation carriers may not only have a higher risk of developing contact allergy to nickel and/or other metals (5), but, in a lifetime perspective, also have an earlier onset of nickel allergy and dermatitis, and a stronger patch test response to nickel sulfate. We therefore set out to investigate whether subjects with *FLG* null mutation status report nickel dermatitis at an earlier age than subjects with wild-type *FLG* status. Furthermore, we investigated whether the strength of patch test reactivity to nickel sulfate was stronger in null mutation carriers than individuals with wild-type *FLG*.

Materials and Methods

Study population

A total of 7931 Danes (age range 18–69 years) in Copenhagen, drawn from the Danish Civil Registration System, were invited to participate in a cross-sectional study; 3471 (43.7%) subjects underwent a general health examination between June 2006 and May 2008 (10). A total of 3460 (99.7%) participants were patch tested, and 3346 (96.4%) were *FLG* genotyped for the two most common Caucasian mutations: R501X and 2282del. More women than men, and more older than younger individuals, participated (mean age: 47.5 years) (11). The study was approved by the Ethics Committee of Copenhagen County (KA-20060011), and a written informed consent form was obtained from all participants.

Questionnaire

A questionnaire concerning general health, lifestyle and socioeconomic factors was sent to the participants with the invitation letter. A description of eczema was included: 'Eczema is an itching skin disorder showing redness, dryness, and possibly vesicles and exudation. Eczema is

present at the same area for some time.' An affirmative answer to the question 'Have you ever had eczema under the buckle of your watch strap, under the button of your jeans, or from wearing ear pins or earrings?' was regarded as a nickel dermatitis event. Age of onset was then defined by the question 'If yes, how old were you at the first occurrence?' Ear piercing status was assessed through the question 'Have you ever had your ears pierced?', and if the answer was affirmative, participants were asked 'If yes, how old were you the first time you had them pierced?' A history of atopic dermatitis was defined according to the UK Working Party's diagnostic criteria as a history of an itchy skin condition plus a minimum of two of four minor criteria (12). Thus, the major criterion was an itchy skin condition, and the minor criteria were: a history of flexural involvement, a history of asthma or hay fever, generalized dry skin within the last year, and onset before the age of 2 years.

Patch testing

Nickel patch testing was performed with the standardized ready to apply TRUE test® (Mekos Laboratories, Hillerød, Denmark). Patch tests with instructions for self-application to the upper back 2 days before presentation for health examination were mailed to the participants. Trained healthcare personnel performed patch test readings, and the reactions were photographed 1–1.5 hr after removal (supervised by J.P.T. and A.L.). The photographs were later reviewed by T.M., N.H.N., A.L. and J.P.T. to confirm that the recommendations of the International Contact Dermatitis Research Group (ICDRG) had been followed (13). Positive allergic reactions were scored as +, ++, or +++, according to the ICDRG criteria. Thus, homogeneous redness and infiltration in the entire test area was scored as a + reaction. Homogeneous redness, infiltration and vesicles in the test area were scored as a ++ reaction, and homogeneous redness, infiltration and coalescing vesicles in the test area as a +++ reaction. In cases where a patch did not have direct skin contact on the patch test reading, or if participants had removed the nickel patch from the test series before application because of known allergy status, data were registered as missing.

FLG genotyping

Blood samples were taken on the day of the health examination, and genomic DNA was purified from white blood cells. Regions covering the two major mutations R501X and 2282del4 were amplified by polymerase chain reaction (PCR) from genomic DNA, and the PCR products were subsequently hybridized to microbeads carrying either mutation-specific or wild-type-specific probes.

Detection was performed on a BioPlex 200 (BioRad, Hercules, CA, USA).

Statistical analysis

Characteristics were compared with the χ^2 -test. A logistic regression analysis was performed with self-reported nickel dermatitis as the dependent variable and with sex, ear piercing status (yes/no), *FLG* genotype (null/wild type) and self-reported atopic dermatitis (yes/no) as the explanatory variables. An interaction term between atopic dermatitis and *FLG* genotype was inserted in a similar regression analysis, to test whether nickel dermatitis depended on *FLG* status in participants with atopic dermatitis. Finally, we performed a logistic regression analysis to confirm that self-reported nickel dermatitis among participants who reported atopic dermatitis was nickel-specific. In this analysis, adjustment was additionally performed for nickel allergy (positive patch test reaction to nickel). Associations were expressed as odds ratios (ORs) with 95% confidence intervals (CIs).

For the different combinations of atopic dermatitis and *FLG* status, we constructed a variable with the following groups: (i) presence of atopic dermatitis and *FLG* null; (ii) presence of atopic dermatitis and *FLG* wild type; (iii) absence of atopic dermatitis and *FLG* null; and (iv) absence of atopic dermatitis and *FLG* wild type. The χ^2 trend test (linear-by-linear association) was used to test for statistically significant differences across the groups of the constructed variable.

The event history distributions were generated using Kaplan–Maier survival statistics. In the analyses, self-reported nickel dermatitis was estimated without the bypass effect. Thus, participants who reported a piercing event were right censored in the analyses, provided that piercing took place before the onset of nickel dermatitis. Participants therefore contributed with risk years from birth and until one of the following events: (i) onset of nickel dermatitis; (ii) first ear piercing event; or (iii) the year of the study, provided that neither of the former events had occurred. The mean onset of nickel dermatitis was calculated with the Kaplan–Meier survival method, including only participants with a history of nickel dermatitis. Owing to an excess of events early in life and a higher proportion of censored observations later in life, the Tarone–Ware significance test was used, and a p -value <0.05 was considered to be significant.

Patterns of patch test reactivity to nickel sulfate (0.20 mg/cm²) for the *FLG* wild-type and null genotypes, respectively, were investigated with the χ^2 -test (linear-by-linear association).

All statistical analyses were performed with SPSS (SPSS, Chicago, IL, USA) for Windows (release 15.0).

Results

Of the 3471 individuals enrolled in the study, 3439 (99.1%) responded to the question on nickel dermatitis. Stratification by sex, *FLG* genotype, ear piercing status and atopic dermatitis is shown in Table 1. More women (55.3%) than men (44.7%) responded. The observed *FLG* genotype frequencies did not deviate significantly from the assumption of Hardy–Weinberg equilibrium (2), and *FLG*_{null} (8.0%) refers to the combined genotypes including both homozygotes and heterozygotes for both null mutations (R501X and 2282del4). More than half of the participants reported ear piercing (53.1%), of whom 85.7% were women (1564/1825).

Among the 3439 respondents, 830 (24.1%) reported nickel dermatitis at some point in their lives [Table 1; 84.7% of women versus 15.3% of men; OR_{CRUDE} 6.50 (95% CI 5.30–7.97)]. *FLG* null mutation status was not associated with a higher risk of reporting nickel dermatitis (OR_{CRUDE} = 1.10, 95% CI 0.82–1.46), whereas a history of ear piercing (OR_{CRUDE} = 4.70, 95% CI 3.91–5.65) and a history of atopic dermatitis (OR_{CRUDE} = 2.40, 95% CI 1.91–3.02) were strongly associated with self-reported nickel dermatitis. Nickel allergy, as an indicator of self-reported nickel-allergic reactions, had, as expected, the strongest association (OR_{CRUDE} = 12.84, 95% CI 9.18–17.95). In a logistic regression analysis with nickel dermatitis as the dependent variable, and sex, atopic dermatitis, ear piercing status and *FLG* mutation status as the independent variables, similar associations were found (Table 1). Further analyses revealed a modest but significant interaction term between *FLG* status and atopic dermatitis ($p = 0.045$). We therefore assessed the effect of the *FLG* genotype in participants who reported atopic dermatitis. In this subgroup, we observed a positive but statistically non-significant association between self-reported nickel dermatitis and the *FLG* null mutation (OR_{ADJUSTED} = 1.65, 95% CI 0.88–3.09, $p = 0.121$). To confirm that the positive association observed for the participants with *FLG* null mutation status was related to nickel allergy, and that it was not just a proxy for increased skin reactivity, the regression analysis was extended to include nickel allergy as an explanatory variable. This analysis revealed a positive association for both nickel allergy (OR_{NICKEL ALLERGY} = 10.56, 95% CI 2.38–46.74, $p = 0.002$) and *FLG* mutation status (OR_{NULL} = 1.47, 95% CI 0.76–2.83, $p = 0.257$).

The prevalence of self-reported nickel dermatitis was further investigated in participants without ear piercings stratified by *FLG* genotype in combination with atopic dermatitis (Table 2). An upward trend was found, from the lowest prevalence among wild-type

Table 1. Characteristics of the 3471 participants from the cross-sectional study in western Copenhagen and their risk of developing nickel dermatitis

		ORs for the explanatory variables Former/latter within variable (95% CI)					
		All, % (n/n _{tot})	Nickel dermatitis ^a , % (n/n _{total})	Crude OR ^b	Adjusted OR ^c		
					All participants (n = 3299)	Participants who reported atopic dermatitis (n = 333)	Participants who reported no atopic dermatitis (n = 2966)
Sex	Women	55.3 (1903/3439)	36.9 (703/1903)	6.50*	4.03*	4.58*	4.03*
	Men	44.7 (1536/3439)	8.3 (127/1536)	(5.30–7.97)	(3.15–5.19)	(1.97–10.63)	(3.09–5.25)
Ear-piercing	Yes	53.1 (1825/3434)	36.0 (657/1825)	4.70*	2.03*	1.41	2.11*
	No	46.9 (1609/3434)	10.7 (172/1609)	(3.91–5.65)	(1.61–2.56)	(0.65–3.05)	(1.65–2.70)
FLG genotype	Null	8.0 (265/3304)	25.7 (68/265)	1.10	0.95	1.65**	0.79
	Wt	92.0 (3039/3304)	23.9 (727/3039)	(0.82–1.46)	(0.70–1.30)	(0.88–3.09)	(0.55–1.15)
Atopic dermatitis ^d	Yes	10.0 (346/3439)	40.8 (141/346)	2.40*	1.84*	—	—
	No	90.0 (3093/3439)	22.3 (689/3093)	(1.91–3.02)	(1.43–2.38)		

OR, odds ratio; CI, confidence interval; Null, carrier of at least one of the two most common null mutations (R501X and 2282del4); Wt, wild-type.

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

**Difference in χ^2 -tests, $p = 0.121$.

^aAn affirmative answer to the question: 'Have you ever had eczema under the buckle of your watch strap, under the button of your jeans, or from wearing ear pins or earrings'?

^bEffect of the noted variable on the development of nickel dermatitis (each separately).

^cEffect of the noted variable on the development of nickel dermatitis in binary regression analyses.

^dDefined by the UK Working Party's diagnostic criteria for atopic dermatitis (one major and at least two of four minor criteria).

Table 2. The prevalence of self-reported nickel dermatitis among participants without ear piercings with stratification for the combination of filaggrin (FLG) genotype and status for atopic dermatitis

	Combined atopic dermatitis and FLG genotype ^a				χ^2 p-trend ^b
	– Atopic dermatitis FLG Wt% (n/n _{total})	– Atopic dermatitis FLG null% (n/n _{total})	+ Atopic dermatitis FLG Wt% (n/n _{total})	+ Atopic dermatitis FLG null% (n/n _{total})	
Proportion reporting nickel dermatitis	9.8 (133/1357)	12.2 (11/90)	17.9 (14/78)	30.0 (6/20)	<0.001

– Atopic dermatitis, absence of atopic dermatitis; + Atopic dermatitis, presence of atopic dermatitis; Wt, wild-type; Null, carrier of at least one of the two most common null mutations (R501X and 2282del4).

^aDivision of the participants in the four denoted groups depending on their FLG genotype status and atopic dermatitis status.

^bLinear-by-linear association.

carriers without atopic dermatitis (9.8%), to null carriers without atopic dermatitis (12.2%), to wild-type carriers with atopic dermatitis (17.9%), to the highest prevalence, which was seen for null carriers with atopic dermatitis (30.0%). The trend was statistically significant (linear-by-linear association, $\chi^2 = 12.14$, $p < 0.001$).

Figure 1 shows the Kaplan–Meier plots, indicating the mean time at risk until the first event of nickel dermatitis (excluding the bypass effect). Even with no effect from ear piercings, women had a significantly shorter mean time at risk than men (Fig. 1a: Tarone–Ware, $\chi^2 = 111.03$, $p < 0.001$), with values of 58.8 years (95% CI 57.4–60.1) and 65.4 years (95% CI 64.8–66.1), respectively. The mean time at risk for the FLG null genotype

was significantly shorter than for the wild-type genotype (Fig. 1b: Tarone–Ware, $\chi^2 = 4.30$, $p = 0.038$), the mean values being 60.3 years (95% CI 57.5–63.0) and 63.3 years (95% CI 62.6–64.0), respectively. When men and women were analysed separately with respect to FLG genotype (Fig. 1c and d), the mean values for FLG null mutants and wild-type genotypes were not significantly different, although the difference was more distinct for men ($\chi^2 = 2.95$, $p = 0.086$) than for women ($\chi^2 = 1.55$, $p = 0.213$).

The mean age at onset (bypass effect excluded) was 21.4 years (95% CI 20.0–22.8) (Table 3). The FLG null genotype consistently showed a lower mean age at onset of nickel dermatitis than the wild-type genotype (Table 3).

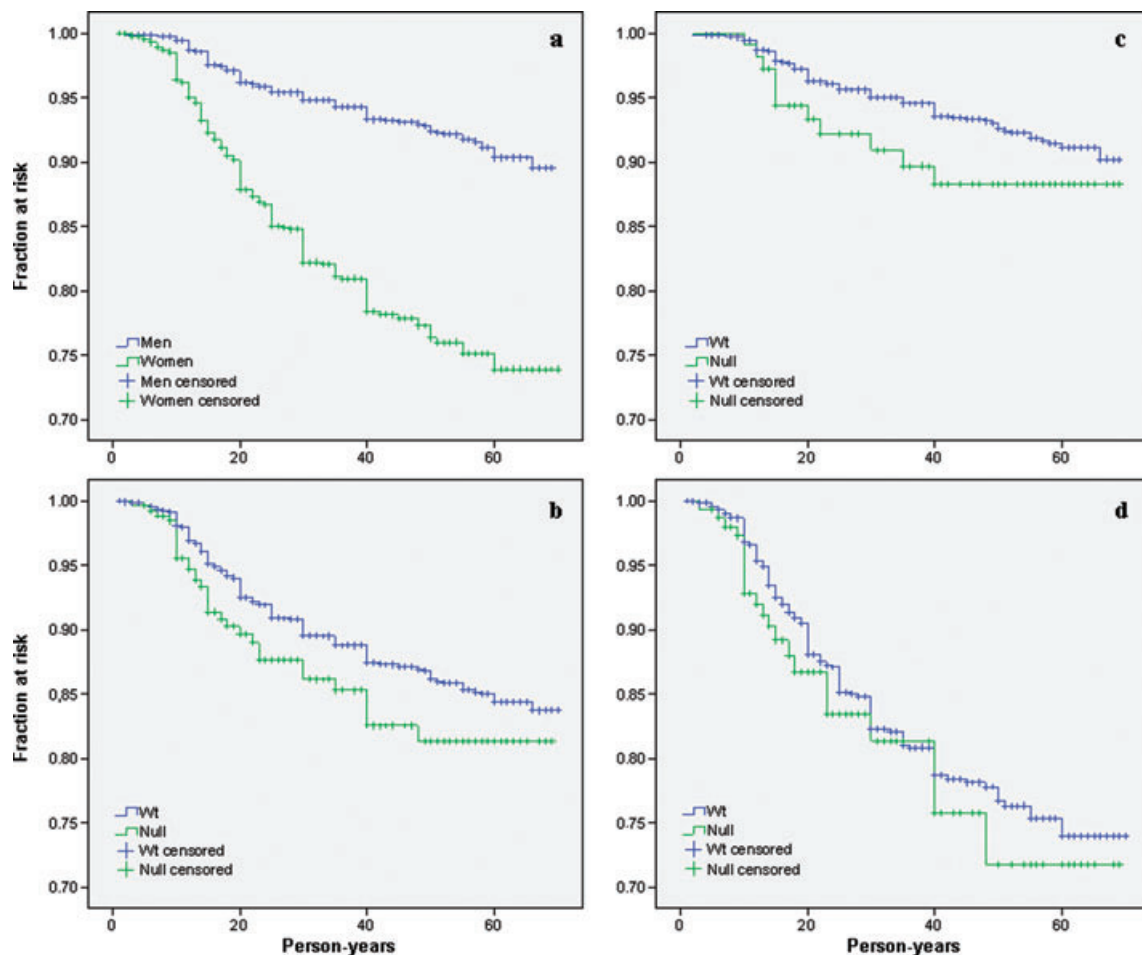


Fig. 1. Kaplan–Meier event history functions. (a) Respondents stratified by sex. (b) Respondents stratified by *FLG* genotype. (c) Male respondents stratified by *FLG* genotype. (d) Female respondents stratified by *FLG* genotype. Person-years: years at risk for each individual until nickel dermatitis debut (event, step down), first ear piercing (censored, +), or age, if neither of the former occurred (censored, +). Wt, wild-type; Null, carrier of at least one of the two most common null mutations (R501X and 2282del4).

Table 3. Mean age at onset (years) of nickel dermatitis stratified by filaggrin (*FLG*) genotype in participants without ear piercings

	Overall (95% CI)	<i>FLG</i> Wt (95% CI)	<i>FLG</i> null (95% CI)	<i>p</i> -value ^a
Total	21.4 (20.0–22.8)	21.7 (20.2–23.3)	18.3 (14.4–22.2)	0.091
Men	25.8 (22.9–28.7)	26.5 (23.3–29.6)	20.6 (14.7–26.6)	0.173
Women	19.4 (17.8–20.9)	19.6 (18.0–21.3)	17.2 (12.1–22.3)	0.172

Wt, Wild-type; Null, carrier of at least one of the two most common null mutations (R501X and 2282del4); CI, confidence interval.

^aComparison of *FLG* wild type with *FLG* null (Tarone–Ware).

To investigate whether the *FLG* null genotypes had an augmented response following skin contact with nickel, the strength of patch test reactivity was analysed with respect to *FLG* genotype (Table 4). Among the 107 participants who had a + reaction, 94.4% of the reactions were accounted for by the *FLG* wild-type genotype. Among the ++ reactions, the proportion of *FLG* wild type was 89.2%, whereas the proportion

among the +++ reactions was only 66.7%. The reverse tendency was observed for the *FLG* null genotype, which accounted for 5.6%, 10.8% and 33.3% of the +, ++ and +++ reactions, respectively. Statistically, the reactivity patterns (relative proportions) of the two genotypes were not significantly different, although a low *p*-value was obtained (linear-by-linear association, $\chi^2 = 3.181$, $p = 0.075$).

Table 4. Patch test reactivity to nickel sulfate (0.20 mg/cm²) stratified by filaggrin (*FLG*) genotype

		<i>FLG</i> Wt%	<i>FLG</i> null%	Total%
Patch test reactivity ^a	+	94.4 (101)	5.6 (6)	100.0 (107)
	++	89.2 (74)	10.8 (9)	100.0 (83)
	+++	66.7 (2)	33.3 (1)	100.0 (3)
χ^2 <i>p</i> -trend ^b		0.075		

Wt, Wild-type; Null, carrier of at least one of the two most common null mutations (R501X and 2282del4).

^aReadings were performed according to the International Contact Dermatitis Research Group criteria: +, homogeneous redness and infiltration in the entire test area; ++, homogeneous redness, infiltration and vesicles in the test area; +++, homogeneous redness, infiltration and coalescing vesicles in the test area.

^bLinear-by-linear association.

Discussion

This study suggested that *FLG* null mutations may increase the risk of self-reported nickel dermatitis in subjects with atopic dermatitis, and also that null mutations may lower the age at onset of nickel dermatitis.

Interestingly, the observed difference in the mean time at risk between *FLG* wild-type and null genotypes was more distinct among men than among women (Fig. 1c and d). The explanation for this finding is uncertain, but we speculate that the difference was stronger for men, as they are generally less exposed to nickel from, for example, jewellery than are women. Thus, the nickel-binding capacity of FLG could be saturated in a larger proportion of women with wild-type *FLG*, resulting in a higher prevalence of nickel dermatitis (as nickel then penetrates the skin). This would tend to dilute the difference between wild-type and null mutation genotypes that can be observed in women. Our findings may support the notion that a certain number of nickel ions can be held in the stratum corneum, the amount being proportional to the amount of FLG present (as well as other nickel-binding proteins). Thus, a lower threshold for nickel sensitization and elicitation is to be expected in subjects with *FLG* null mutation status. The merged curves (Fig. 1d) could reflect such a heavy degree of cutaneous exposure to nickel among women in their early 20s to age 40 years. Thus, at lower exposure levels, the curves would be distinctly separated with respect to *FLG* genotype (as seen for men; Fig. 1c). This might be the future scenario, when the cohort effect, resulting from individuals sensitized before the nickel legislation in Denmark and Europe, disappears (14, 15). The mean and median debut year for nickel dermatitis among non-pierced individuals in the present study was 1977 (data not shown).

Data on participants without ear piercings may be used to evaluate the role of FLG as the barrier against nickel sensitization through intact skin. Data on participants who are ear pierced represent a combination of scenarios, where FLG's role is to protect the individual against sensitization but also elicitation. Alternatively, the effect of FLG may be undermined if sensitization and elicitation happen both as a result of, and at the site of, the piercing. Analyses support the hypothesis that ear piercings obscure the FLG effect in association analyses with nickel allergy. In an event history analysis, where the bypass effect was disregarded, the female genotype curves (corresponding to Fig. 1d) were coincident (data not shown).

The mean age at onset of nickel dermatitis among non-pierced participants also supports the possible association between *FLG* null mutation status and nickel dermatitis, as a lower age was consistently seen for the *FLG* null genotype (Table 3). The observed statistical insignificance is probably attributable to inadequate statistical power. Disregarding the bypass effect, the mean age at onset was 22.1 years (95% CI 21.2–22.9) (data not shown). This is reminiscent of the results from a Finnish study of the general population in the late 1970s, where Peltonen et al. found that the mean age of onset for nickel contact dermatitis was 23.1 years (16). The similarity in mean age estimates probably reflects a cohort effect resulting from the large proportion of Danes who experienced nickel allergy and dermatitis during the 1970s and 1980s. Thus, if only young subjects had been questioned, the mean age could possibly have changed, as the frequency of ear piercing, age at first piercing and fashion trends may have changed markedly over the three decades.

Patch test reading data suggested an association between the *FLG* null genotype and a lower threshold to nickel, as the proportion of null mutants among positive patch test readings (+, ++, and +++) increased with increasing reaction strength (Table 4). The +++ data should, however, be interpreted with caution, considering the low counts. A dose–response relationship apparently exists, and may be registered in conventional patch testing. The amount of nickel used for the purpose of patch testing is much higher than the amount of nickel (per area) that an individual will be exposed to in our environment. The results presented in Table 4 indicate that *FLG* null carriers have a lower elicitation threshold than wild-type individuals, and the finding could therefore prove to be of clinical significance.

The two null mutations R501X and 2282del4 examined in our study were previously found to be strong predisposing factors for atopic dermatitis (17). We found a significant interaction term between *FLG* genotype and atopic dermatitis in the regression analysis with nickel

dermatitis as the dependent variable (Table 1). In terms of the development of nickel dermatitis, this result means that *FLG* deficiency may increase the risk of self-reported nickel dermatitis in subjects with atopic dermatitis. Crude data analysis suggested that the prevalence of self-reported nickel dermatitis among individuals without ear piercings was dependent on *FLG* status in combination with atopic dermatitis (Table 2). The largest proportion was registered within *FLG* null mutation carriers with atopic dermatitis (30.0%), followed by *FLG* wild-type carriers with atopic dermatitis (17.9%), *FLG* null genotype carriers without atopic dermatitis (12.2%), and finally *FLG* wild-type carriers without atopic dermatitis (9.8%).

The relationship between nickel sensitivity and atopic dermatitis is not clear (5, 18–20), but previous studies may have been confounded by atopic dermatitis subpopulations with respect to *FLG* genotype. We previously found that the presence of *FLG* null mutations increased the risk of self-reported hand eczema among adult Danes with atopic dermatitis (21). Thus, it appears that atopic participants with null mutations have increased skin reactivity in general. Nevertheless, there was evidence in support of the association between *FLG* null mutation status and nickel dermatitis being, to a large degree, explained by increased nickel sensitivity, as nickel allergy was significantly associated with nickel dermatitis in the regression analysis (Table 1). Because of small groups, it

was not possible to stratify by atopic dermatitis in the risk time analyses (Fig. 1; Table 3). Such analyses would be interesting to perform on suitable data.

In this study, an affirmative answer to the question ‘Have you ever had eczema under the buckle of your watch strap, under the button of your jeans, or from wearing ear pins or earrings?’ could potentially include reactions towards other metals such as cobalt or chromate, or be a marker of irritation. The validity of self-reported nickel allergy has been questioned in a Swedish study, where they found some discrepancies (22). However, nickel is the most prevalent metal allergen (23), and, in a number of earlier studies, the majority of cases of self-reported metal dermatitis were associated with nickel allergy (24–28). In our analysis, 76.5% of the participants who were found to be allergic to nickel in patch testing also reported nickel dermatitis (Table 1). We therefore consider our results to primarily reflect nickel-allergic reactions, but acknowledge that misclassification may be present to a small degree.

In conclusion, *FLG* null mutations may lower the age of onset of nickel dermatitis. The hypothesis that ear piercings obscure the effect of *FLG* null mutations on the development of nickel allergy in statistical analyses was supported. An association between the null genotype and increased nickel sensitivity was indicated by patch test reading and questionnaire data.

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