

The association between null mutations in the filaggrin gene and contact sensitization to nickel and other chemicals in the general population

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Summary

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Background It was recently shown that filaggrin gene (FLG) null mutations are positively associated with nickel sensitization. We have hypothesized that histidine-rich filaggrin proteins in the epidermis chelate nickel ions and prevent their skin penetration and exposure to Langerhans cells. Furthermore, we have proposed that the low degree of genetic predisposition to nickel sensitization found by a Danish twin study was explained by a high prevalence of ear piercing among participants resulting in 'bypassing' of the filaggrin proteins.

Objectives To investigate the association between FLG null mutations and (nickel) contact sensitization.

Methods A random sample of 3335 adults from the general population in Denmark was patch tested and genotyped for R501X and 2282del4 in the FLG gene.

Results The combined carrier frequency of FLG null mutations was 8.1%. Nickel, fragrance and contact sensitization to at least one allergen were not associated with FLG null mutations. A crude analysis on women who did not have ear piercings revealed a positive association between FLG null mutations and nickel sensitization [8.3% vs. 2.4%; odds ratio (OR) 3.71, 95% confidence interval (CI) 0.73–18.96] as well as between FLG null mutations and allergic nickel dermatitis (8.3% vs. 1.3%; OR 6.75, 95% CI 1.17–38.91). FLG mutation status and atopic dermatitis were positively associated with neomycin or ethylenediamine sensitization.

Conclusions This study suggests that FLG null mutations may be a risk factor for the development of nickel sensitization. However, ear piercing was a much stronger risk factor in our general population and we could therefore identify a positive association only in women without ear piercings. Contact sensitization to specific chemicals is related to treatment exposure.

Contact sensitization is prevalent, affecting 10–20% of the general population.^{1,2} Risk factors include female sex, ear piercing and perhaps tobacco smoking.^{1,3–6} Genetic predisposition to nickel sensitization has previously been investigated in two Danish twin studies: Menné and Holm⁷ found that genetic factors influenced the development of nickel sensitization as it was more common in monozygotic than dizygotic twins, whereas Bryld *et al.*⁸ found that nickel sensitization could be explained largely by environmental factors and only to a lesser degree by genetic factors. Thus, it was surprising when Novak *et al.*⁹ recently showed that the two most fre-

quent null mutations in the filaggrin gene (FLG), R501X and 2282del4, were positively associated with nickel sensitization [odds ratio (OR) 1.91, 95% confidence interval (CI) 0.91–4.04], and significantly associated with nickel sensitization in combination with self-reported intolerance to fashion jewellery (OR 4.04, 95% CI 1.35–12.06). Interestingly, the authors did not find any association with fragrance sensitization or contact sensitization to at least one allergen.

We recently hypothesized that filaggrin, a histidine-rich protein found in the upper layers of the epidermis, could chelate nickel and prevent its penetration through the skin,^{10–12} and

that the contradictory results obtained in the two Danish twin studies^{7,8} could possibly be explained by different routes of nickel exposure in female participants.¹² Thus, in the more recent twin study by Bryld *et al.*,⁸ nickel sensitization was likely to occur following ear piercing in the majority of women (ear piercing would result in direct nickel ion exposure to Langerhans cells without previous skin penetration and remove the effect of FLG null mutations in data analyses).¹² In the older twin study by Menné and Holm,⁷ primary nickel exposure was mostly related to cutaneous contact with metallic suspenders and buttons rather than ear piercing. Nickel sensitization would therefore occur following penetration of nickel ions through the skin and the effect of FLG could be observed.

The present study aimed to reinvestigate the association between FLG null mutations and nickel sensitization and contact sensitization. A random sample of adults from the general population in Copenhagen was invited to participate in a general health examination including patch testing and FLG genotyping. The current study is of relevance because FLG null mutations are prevalent⁹ and an association with nickel or contact sensitization could have clinical implications.

Materials and methods

Study populations

Between June 2006 and May 2008, a cross-sectional study was performed in the general population in Copenhagen, the capital of Denmark. 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 (43.8%) subjects participated in a general health examination, 3460 (99.7%) were patch tested and 3346 (96.4%) were genotyped for FLG mutations 2282del4 and R501X. The participation rate was higher among older age groups than among younger age groups in both sexes.¹³ 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 about health, lifestyle and socioeconomic factors. 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'. Among a broad variety of questions on general health and lifestyle, participants were asked 'has your doctor ever told you that you suffered from asthma?', 'has your doctor ever told you that you suffered from rhinitis?', 'have you ever experienced skin redness, a rash and itching caused by exposure to cosmetics, moisturizers, lotions or such related products?' (an affirmative answer was used to define cosmetic dermatitis) and 'have you ever had eczema under the tightener

of your watch strap, under the button of your jeans or from wearing ear pins or earrings?' (an affirmative answer together with a positive patch test reaction to nickel sulphate was used to define allergic nickel dermatitis) and 'have you ever had your ears pierced?'. Finally, 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.¹⁴ The major criterion was an itchy skin condition and 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.¹⁴

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). The haptens in the TRUE-test differed from the European baseline series by inclusion of thiomersal and exclusion of primine, sesquiterpene lactone mix, tixocortol pivalate, budesonide, hydroxyisohexyl-3-cyclohexene carboxaldehyde, methylidibromo glutaronitrile and fragrance mix II. Furthermore, the TRUE-test contained black-rubber mix instead of N-isopropyl-N'-phenyl-para-phenylenediamine, and caine mix (tetracaine, benzocaine and dibucaine) instead of benzocaine. 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.T. and A.L.). Photographs were reviewed by T.M., N.H.N., A.L. and J.P.T. This was done to ensure that the International Contact Dermatitis Research Group (ICDRG) criteria were used consistently.¹⁵ Contact sensitization was defined as a positive (at least grade 1+ according to the ICDRG) patch test to at least one allergen or mixes of haptens. In case the patch had no skin contact upon patch test reading, or if the subject had removed it prior to testing as a result of known contact sensitization, it was regarded as missing data.

Measurement of IgE antibodies

Venous blood was taken on the day of examination and was left to coagulate for 2 h. The serum was then separated by centrifugation at 3000 g for 10 min and frozen immediately afterwards. The serum samples were analysed for IgE specific to birch, grass (timothy), cat and mite (*Dermatophagoides pteronyssinus*) with the ADVIA Centaur IgE antibody assay system (Bayer Corporation, Spokane, WA, U.S.A.).^{16,17} The analysis for IgE antibodies was judged to be positive if the measurement was in excess of 0.35 kU L⁻¹. Specific IgE positivity was defined as a positive test to at least one of the four allergens tested.

Filaggrin 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 probes attached to paramagnetic beads (Luminex, Austin, TX, U.S.A.) and analysed on a BioPlex 200 (Biorad, Hercules, CA, U.S.A.).

Statistical analysis

Characteristics of participants were compared using the χ^2 test. Due to a well-known marked sex difference in the prevalence of sensitization to contact allergens, the analyses were stratified by sex, age and ear-piercing status. Deviation from the Hardy–Weinberg equilibrium was tested using the free online calculator at the Online Encyclopedia for Genetic Epidemiology studies (<http://www.oege.org/software/hwe-mr-calc.shtml>) for both FLG null mutations.

A logistic regression model was performed with allergic nickel dermatitis as the dependent variable, and with sex, age group ('18–35 years', '36–55 years', '56–69 years'), nickel sensitization ('no', 'yes'), ear-piercing status ('no', 'yes'), atopic dermatitis ('no', 'yes') and FLG filaggrin mutation status ('wild-type', 'null mutation') as the independent variables. In this model, a test for interaction between nickel sensitization and FLG mutation status was performed by using a log-likelihood ratio test. Furthermore, a logistic regression analysis was performed with 'nickel sensitization' as the dependent variable and with the following independent variables: sex, age group ('18–35 years', '36–55 years', '56–69 years'), ear-piercing status ('no', 'yes') and FLG mutation status ('wild-type', 'null mutation'). In this model, a test for interaction between ear-piercing status and FLG mutation status was performed by using a log-likelihood ratio test. Logistic regression analyses similar to those described above were also performed for 'women' and for 'women who were not ear pierced'. Furthermore, a logistic regression analysis similar to the analysis for nickel allergy was performed with 'contact sensitization to at least one allergen' as the dependent variable. Finally, a logistic regression analysis was performed with 'neomycin or ethylenediamine sensitization' as the dependent variable and with sex, age group ('18–35 years', '36–55 years', '56–69 years'), FLG mutation status ('wild-type', 'null mutation') and atopic dermatitis ('no', 'yes') as the independent variables.

Associations were expressed as ORs with 95% CI. The Kolmogorov–Smirnov test was used to assess the normal distribution of age. Data analyses were performed using SPSS (SPSS Inc., Chicago, IL, U.S.A.) for Windows (release 15.0).

Results

Characteristics of the participants are shown in Table 1. The age of the study population was not normally distributed as assessed by the Kolmogorov–Smirnov test ($P < 0.001$). The median age was 48 years [interquartile range (IQR) 19], 49 years (IQR 20) for men and 48 years (IQR 20) for women. 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 null mutations. The observed genotype prevalences of both polymorphisms did not deviate statistically significantly ($P > 0.05$) from the expected prevalences under the Hardy–Weinberg equilibrium assumption. FLG mutation status among participants is presented in Table 2. The combined carrier frequency was 8.1%.

Stratification by FLG mutation status revealed that the prevalence of self-reported atopic dermatitis was significantly higher among participants with null mutations in the FLG gene than among participants without mutations (21.8% vs. 9.1%) (Table 1). This positive association was found in both men and women (Table 3). A possible association between contact sensitization and FLG null mutation status was investigated. We found that nickel sensitization, fragrance sensitization and contact sensitization to at least one allergen were not associated with FLG null mutations in the entire population (Table 1). When association analysis was performed on the remaining TRUE-test allergens with lower prevalences,² we found that most contact allergies were evenly distributed among participants with and without FLG null mutations (data not shown). Exceptions were ethylenediamine and neomycin sensitization which differed significantly between the groups (Table 1). Overall, no positive associations were found for FLG null mutation status on the one hand and self-reported cosmetic dermatitis and allergic nickel dermatitis on the other hand. Stratification by age group did not change the outcome of our crude data analyses. Stratification by sex showed that contact sensitization to at least one allergen was unevenly distributed among women with and without null mutations (18.5% vs. 13.6%) and that it was mainly explained by different prevalences of ethylenediamine and neomycin sensitization (Table 3).

We investigated the possible association between nickel sensitization or allergic nickel dermatitis and FLG null mutations among subjects who did not report ear piercing (Tables 1 and 3). The analyses revealed that among female participants without ear piercing, FLG null mutation status was positively but not significantly associated with nickel sensitization (8.3% vs. 2.4%, OR 3.71, 95% CI 0.73–18.96) and positively and significantly associated with allergic nickel dermatitis (8.3% vs. 1.3%, OR 6.75, 95% CI 1.17–38.91). Of note, the prevalence of atopic dermatitis was 13.4% in participants who had their ears pierced and 6.4% in participants who did not have their ears pierced (for women these values were 14.5% and 6.6%, respectively). Thus, the association between FLG null mutations and nickel allergy in those without ear piercings was not explained by an over-representation of atopic dermatitis among subjects without ear piercings.

We evaluated whether or not it could be assumed that the effect of FLG null mutation status was independent of nickel sensitization. Thus, a logistic regression analysis was performed with allergic nickel dermatitis as the dependent variable and with the following explanatory variables: sex, age group, atopic dermatitis, FLG mutation status and nickel sensitization. In this model, a test for interaction between nickel sensitization and FLG mutation status was performed by using a log-likelihood ratio test.

Table 1 Characteristics of patch-tested participants stratified by null mutations in FLG: null alleles R501X and 2282del4

	All % (n/n _{total})	FLG null mutation ^a		Odds ratio (95% CI) ^b
		Yes % (n/n _{total})	No % (n/n _{total})	
Men	44.7 (1491/3335)	41.6 (112/269)	45.0 (1379/3066)	0.87 (0.68–1.12)
Women	55.3 (1844/3335)	58.4 (157/269)	55.0 (1687/3066)	1.15 (0.89–1.48)
Atopic dermatitis ^c	10.4 (324/3202)	21.8 (56/257)	9.1 (268/2945)	2.78 (2.02–3.83)*
Asthma	10.9 (356/3280)	13.3 (35/263)	10.6 (321/3017)	1.29 (0.89–1.87)
Rhinitis	18.0 (590/3273)	18.3 (48/262)	18.0 (542/3011)	1.02 (0.74–1.42)
Specific IgE ^d	23.4 (775/3312)	22.4 (60/268)	23.5 (715/3044)	0.94 (0.69–1.26)
Contact sensitization to at least one allergen	9.8 (328/3335)	12.3 (33/269)	9.6 (295/3066)	1.31 (0.89–1.93)
Nickel sensitization (all respondents)	6.1 (195/3213)	6.2 (16/258)	6.1 (179/2955)	1.03 (0.61–1.74)
Nickel sensitization (respondents without ear-piercing)	1.1 (17/1507)	1.9 (2/105)	1.1 (15/1402)	1.80 (0.41–7.96)
Fragrance mix I sensitization	1.6 (53/3249)	1.5 (4/261)	1.6 (49/2988)	0.93 (0.33–2.61)
Ethylenediamine sensitization	0.2 (8/3218)	1.2 (3/255)	0.2 (5/2963)	7.04 (1.67–29.64)*
Neomycin sensitization	0.1 (2/3276)	0.4 (1/265)	0.03 (1/3011)	11.40 (0.71–182.80)
Allergic nickel dermatitis ^e (all respondents)	4.4 (147/3333)	4.8 (13/269)	4.4 (134/3064)	1.11 (0.62–1.99)
Allergic nickel dermatitis ^e (respondents without ear piercing)	0.6 (9/1551)	1.8 (2/111)	0.5 (7/1440)	3.76 (0.77–18.30)
Dermatitis from a cosmetic product ^f	47.3 (1555/3291)	50.9 (135/265)	46.9 (1420/3026)	1.17 (0.91–1.51)

^aGenotyping was done for two common null mutations in the FLG gene (R501X and 2282del4). ^bOdds ratio (OR) with 95% confidence intervals (CI) for the comparison of patients with and without null mutations in the FLG gene. ^cAtopic 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.¹⁴ ^dAnalysis for IgE specific to birch, grass (timothy), cat and mite (*Dermatophagoides pteronyssinus*); the analysis was judged to be positive if the measurement was in excess of 0.35 kU L⁻¹. ^eAn affirmative answer to the question 'have you ever had eczema under the tightener of your watch strap, under the button of your jeans or from wearing ear pins or earrings?' together with a positive patch test reaction to nickel sulphate. ^fAn affirmative answer to the question 'have you ever experienced skin redness, a rash and itching caused by exposure to cosmetics, moisturizers, lotions or such related products?'. *Significant difference.

likelihood ratio test. The analysis revealed an insignificant P-value of the interaction term ($P = 0.99$). The result means that the possible effect of FLG null mutations on the prevalence of allergic nickel dermatitis did not differ significantly between those with or without nickel sensitization. A similar logistic regression analysis was performed in female participants but it did not reveal any association between nickel allergy and FLG null mutation status (Table 4). An adjusted logistic regression analysis in female participants without ear piercing gave empty strata and was therefore not shown.

Two logistic regression analyses with nickel sensitization and contact sensitization to at least one allergen as the dependent variables, respectively, and with sex, age group, ear-piercing status and FLG mutation status as the independent variables showed that FLG null mutation status was not significantly associated with nickel sensitization (Table 5) and

contact sensitization (data not shown). Also, the interaction term between FLG mutation status and ear-piercing status was $P = 0.36$. Thus, the effect of FLG null mutations on the prevalence of nickel allergy did not depend significantly on ear-piercing status. However, in a similar logistic regression analysis including only female participants, the interaction term between FLG mutation status and ear piercing was $P = 0.08$. The outcome of a logistic regression analysis performed in female participants is shown in Table 5. Finally, a logistic regression analysis was performed with 'neomycin or ethylenediamine sensitization' as the dependent variable and with sex, age group, FLG mutation status and atopic dermatitis as the independent variables (Table 6). It showed that both FLG mutation status and atopic dermatitis were positively associated with neomycin or ethylenediamine sensitization.

		2282del4 % (n)		
		Wild-type	Heterozygote	Homozygote
R501X % (n)	Wild-type	91.9 (3066)	4.5 (151)	0.1 (3)
	Heterozygote	3.3 (109)	0.1 (2)	0.03 (1)
	Homozygote	0.1 (3)	0	0

Table 2 Null mutation status of R501X and 2282del4 among 3335 patch-tested participants

Table 3 Characteristics of male and female participants stratified by FLG mutation status

	FLG null mutation ^a					
	Men			Women		
	Yes % (n)	No % (n)	Crude OR with 95% CI ^b	Yes % (n)	No % (n)	Crude OR with 95% CI ^b
Atopic dermatitis ^c	18.1 (19/105)	5.4 (72/1323)	3.84 (2.21–6.66)*	24.3 (37/152)	12.1 (196/1622)	2.34 (1.57–3.49)*
Asthma	11.1 (12/108)	8.1 (110/1352)	1.41 (0.75–2.66)	14.8 (23/155)	12.7 (211/1665)	1.20 (0.75–1.91)
Rhinitis	16.7 (18/108)	17.3 (234/1355)	0.96 (0.57–1.62)	19.5 (30/154)	18.6 (308/1656)	1.06 (0.70–1.61)
Specific IgE ^d	28.6 (32/112)	27.4 (374/1364)	1.06 (0.69–1.62)	17.9 (28/156)	20.3 (341/1680)	0.86 (0.56–1.32)
Contact sensitization to at least one allergen	3.6 (4/112)	4.7 (65/1379)	0.75 (0.27–2.10)	18.5 (29/157)	13.6 (230/1687)	1.44 (0.94–2.20)
Nickel sensitization (all respondents)	0 (0/104)	1.00 (14/1335)	–	10.4 (16/154)	10.2 (165/1620)	1.00 (0.94–1.06)
Nickel sensitization (respondents without ear piercing)	0 (0/81)	0.7 (8/1109)	–	8.3 (2/24)	2.4 (7/293)	3.71 (0.73–18.96)
Fragrance mix I sensitization	1.8 (2/109)	1.4 (19/1348)	1.31 (0.30–5.69)	1.3 (2/152)	1.8 (30/1640)	0.72 (0.17–3.02)
Ethylenediamine sensitization	0 (0/106)	0.1 (2/1343)	–	2.0 (3/149)	0.2 (3/1620)	1.02 (1.00–1.04)*
Neomycin sensitization	0 (0/112)	0 (0/1357)	–	0.7 (1/153)	0.1 (1/1654)	10.88 (0.68–174.73)
Allergic nickel dermatitis ^e (all respondents)	0 (0/112)	0.4 (6/1379)	–	8.3 (13/157)	7.6 (128/1685)	1.10 (0.61–1.99)
Allergic nickel dermatitis ^e (respondents without ear piercing)	0 (0/87)	0.3 (3/1139)	–	8.3 (2/24)	1.3 (4/301)	6.75 (1.17–38.91)*
Ear piercing	21.6 (24/111)	16.6 (226/1365)	1.39 (0.86–2.23)	84.6 (132/156)	82.0 (1372/1673)	1.21 (0.77–1.90)
Dermatitis from a cosmetic product ^f	40.9 (45/110)	33.7 (457/1360)	1.37 (0.92–2.03)	58.1 (90/155)	57.7 (963/1666)	1.01 (0.72–1.41)

^aGenotyping was done for two common null mutations in the FLG gene (R501X and 2282del4). ^bOdds ratio (OR) with 95% confidence intervals (CI) for the comparison of patients with and without null mutations in the FLG gene. ^cAtopic 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.¹⁴ ^dAnalysis for IgE specific to birch, grass (timothy), cat and mite (*Dermatophagoides pteronyssinus*); the analysis was judged to be positive if the measurement was in excess of 0.35 kU L⁻¹. ^eAn affirmative answer to the question 'have you ever had eczema under the tightener of your watch strap, under the button of your jeans or from wearing ear pins or earrings?' together with a positive patch test reaction to nickel sulphate. ^fAn affirmative answer to the question 'have you ever experienced skin redness, a rash and itching caused by exposure to cosmetics, moisturizers, lotions or such related products?'. *Significant difference.

Discussion

This study showed that the combined carrier frequency of the two most frequent null mutations in the FLG gene, R501X and 2282del4, was prevalent among subjects from the general population in Denmark (8.1%). Furthermore, null mutations were strongly associated with atopic dermatitis, which was in accordance with previous reports.^{18–22} The present study is of interest as it is the largest general population study in which genotyping for R501X and 2282del4 mutations has been performed in combination with patch testing. This allowed us to investigate whether or not null mutations in the FLG gene increase the risk of developing contact sensitization. Our analyses showed that no association could be identified between null mutations in the FLG gene and contact sensitization to at least one allergen, nickel sensitization, fragrance mix I sensi-

zation or cosmetic dermatitis in the overall population. However, when we performed a subanalysis on female participants without ear piercings, a positive association was found between FLG null mutation status and nickel sensitization and allergic nickel dermatitis, respectively (Tables 3–5). As ear piercing is highly prevalent among Danish women, the analyses had low power. Nevertheless, the associations were strong enough to support our earlier hypothesis on the association between nickel sensitization and FLG null mutation status (i.e. histidine-rich filaggrin proteins in the epidermis chelate nickel ions and prevent their skin penetration and exposure to Langerhans cells, and that the low degree of genetic predisposition to nickel sensitization found by a relatively recent Danish twin study⁸ could be explained by a high prevalence of ear piercing among participants resulting in 'bypassing' of the filaggrin proteins).¹² Also, our findings support the results

Table 4 Logistic regression analyses with the outcome 'allergic nickel dermatitis' and with different explanatory variables performed in 3335 patch-tested and FLG genotyped subjects (R501X and 2282del4)

Explanatory variable	Adjusted OR with 95% CI ^a	
	All participants	Female participants
FLG null mutations		
No	1 (reference)	1 (reference)
Yes	1.03 (0.57–1.89)	1.08 (0.59–1.98)
Sex		
Male	1 (reference)	–
Female	8.91 (3.67–21.62)	–
Age		
18–35 years	1 (reference)	1 (reference)
36–55 years	1.37 (0.89–2.13)	1.44 (0.92–2.26)
56–69 years	0.52 (0.27–0.94)	0.53 (0.29–0.97)
Ear piercing		
No	1 (reference)	1 (reference)
Yes	4.23 (2.01–8.87)	4.27 (1.85–9.86)
Atopic dermatitis ^b		
No	1 (reference)	1 (reference)
Yes	0.73 (0.42–1.25)	0.74 (0.43–1.28)

^aMutually adjusted for variables shown in the table. ^bAtopic 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.¹⁴ OR, odds ratio; CI, confidence interval.

from a German general population study which found a positive association between nickel sensitization and FLG null mutation status.⁹ Finally, a recent study showed that the level of histidine in the stratum corneum was significantly reduced in patients with null mutations in the FLG gene in comparison to patients with wild-type status.²³

Table 5 Logistic regression analyses with the outcome 'nickel sensitization' and with different explanatory variables performed in 3335 patch-tested and FLG genotyped subjects (R501X and 2282del4)

Explanatory variable	Adjusted OR with 95% CI ^a	
	All participants	Female participants
FLG null mutations		
No	1 (reference)	1 (reference)
Yes	0.90 (0.52–1.55)	0.97 (0.55–1.71)
Sex		
Male	1 (reference)	–
Female	5.34 (2.88–9.90)	–
Age		
18–35 years	1 (reference)	1 (reference)
36–55 years	1.19 (0.82–1.72)	1.15 (0.77–1.70)
56–69 years	0.51 (0.31–0.83)	0.48 (0.28–0.81)
Ear piercing		
No	1 (reference)	1 (reference)
Yes	3.58 (2.02–6.35)	3.77 (1.88–7.52)

^aMutually adjusted for variables shown in the table. OR, odds ratio; CI, confidence interval.

Table 6 Logistic regression analysis with the outcome 'neomycin or ethylenediamine sensitization' and with different explanatory variables performed in 3335 patch-tested and FLG genotyped subjects (R501X and 2282del4)

Explanatory variable	Adjusted OR with 95% CI ^a
FLG null mutations	
No	1 (reference)
Yes	4.94 (1.12–21.77)
Sex	
Male	1 (reference)
Female	1.91 (0.38–9.74)
Age	
18–35 years	1 (reference)
36–55 years	0.70 (0.12–4.31)
56–69 years	1.38 (0.22–8.77)
Atopic dermatitis ^b	
No	1 (reference)
Yes	6.67 (1.54–28.85)

^aMutually adjusted for variables shown in table. ^bAtopic 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.¹⁴ OR, odds ratio; CI, confidence interval.

Although our main finding needs to be reconfirmed, it seems that FLG null mutations may truly increase the risk of developing nickel allergy. However, it should be underscored that the prevalence of nickel sensitization in men was low indicating that environmental nickel exposure needs to be substantial in order to sensitize a subject. Of note, the EU Nickel Directive may change the relative importance of ear piercing and FLG gene mutations. We recently showed that regulatory intervention has decreased the prevalence of nickel allergy among women who had their ears pierced after the Danish nickel regulation was introduced when compared with the prevalence in women who had their ears pierced before its introduction.⁵ Thus, the decreased risk of nickel sensitization from ear piercing and skin contact with jewellery may increase the importance of FLG mutations.

A positive association between FLG null mutation status and contact sensitization to ethylenediamine and neomycin was found (Tables 1 and 3). Ethylenediamine is a sensitizer used in topical antibiotic and corticosteroid creams. Neomycin is an antibacterial that is used widely in topical creams, eye preparations and eardrops. Neomycin is also found in combination preparations with other antibacterials and corticosteroids. 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 ethylenediamine and neomycin. We found that contact sensitization to these ingredients was associated with self-reported atopic dermatitis as well as FLG null mutation status in an adjusted analysis (Table 6). This finding shows that contact sensitization to specific chemicals is related to treatment exposure.

Our study had some weaknesses. Firstly, we performed patch test readings only on day 2. We have previously estimated that 18–29% of positive patch test reactions to nickel sulphate are missed when readings are performed only on day 2 and not also on day 4.²⁴ However, we have no reason to believe that subjects with FLG null mutations are more prone to be late reactors than subjects without mutations although this would tend to bias the results. Furthermore, we performed genotyping for only the two most prevalent FLG null mutations, R501X and 2282del4, which cover approximately 85% of null mutations in the FLG gene among Caucasians.²⁵ It is possible that the other null mutations in the FLG gene were unevenly distributed between those with and without nickel sensitization. Thus, the positive associations we found in this study would probably tend to be stronger if we had also genotyped for the remaining FLG gene mutations. Finally, the participation rate was low (39.1% for men and 48.4% for women).¹³ Participants were informed in the invitation letter that testing for nickel sensitization was part of the health examination. Assuming that a lower participation rate results in a stronger selection of nickel-sensitized participants (i.e. a larger difference in prevalence of nickel sensitization between participants and nonparticipants), this would tend to overestimate the prevalence of FLG null mutations in the general population.

In conclusion, this study suggests that FLG null mutations may be a risk factor for the development of nickel sensitization. However, ear piercing was a much stronger risk factor in our general population and we could therefore identify a positive association only in women without ear piercings.

What's already known about this topic?

- One study has shown that nickel allergy is associated with FLG null mutations (Novak *et al.*, 2008⁹)

What does this study add?

- This study shows that ear piercing is a much greater risk factor for nickel allergy than FLG null mutations
- More importantly, it shows that FLG null mutations are mainly of importance in those without ear piercing
- Finally, it shows that contact allergy to ingredients in topical therapeutics is increased in subjects with atopic dermatitis

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