

The nickel dose–response relationship by filaggrin genotype (*FLG*)

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Summary

Background. On skin contact, nickel accumulates in the stratum corneum, where it is probably bound to proteins and amino acids. One probable contributor is filaggrin, which binds nickel avidly. Filaggrin gene (*FLG*) null mutations lead to a complete lack of filaggrin production from the affected allele, and have been associated with an increased risk of nickel contact sensitization in German and Danish adults.

Objectives. To investigate whether the experimental nickel elicitation threshold level differed between heterozygous *FLG* mutation and non-mutation carriers.

Method. Thirteen nickel-sensitized female patients, seven heterozygous mutation carriers and six non-mutation carriers (genotyped for R501X, 2282del4, or R2447X), were patch tested and performed a repeated open application test (ROAT) with a nickel sulfate dilution series. Logistic threshold dose–response analyses were used to test for differences between the two groups.

Results. No difference was found in the dose–response relationship between *FLG* mutation and non-mutation carriers.

Conclusions. On the basis of this small patient study, it appears that the elicitation threshold level for nickel is independent of *FLG* null mutation single-allele carrier status.

Key words: experimental nickel contact elicitation; filaggrin genotype.

Skin contact with metal alloys that contain and release nickel is very frequent. Whereas some contacts are prolonged (e.g. with jewellery, watches, and buttons), others are short-term and intermittent (e.g. with door knobs, keys, and coins). When nickel is released and deposited on the skin, it may indeed permeate into the deeper and viable layers of the epidermis, and cause contact sensitization. However, initially, nickel tends to accumulate in the stratum corneum, presumably being chelated by proteins and amino acids (1, 2).

Filaggrin is an abundant histidine-rich, epidermal protein that is degraded to its constituent amino acids in the stratum corneum (3). The 'natural moisturizing factors' are essential contributors to the hygroscopic properties of the stratum corneum. Importantly, filaggrin provides ~50% of its content via its metabolites, the remaining factors consisting of sodium and chloride ions, lactate, and urea (4). Skin with reduced levels of filaggrin is therefore characterized by xerosis and an increased risk of inflammation, typically atopic dermatitis (3). Moreover, when the epidermis has reduced levels of or no filaggrin and its degradation products, the penetration of nickel ions is probably increased, owing to compromised nickel chelation (5, 6). The skin might also be more permeable in carriers of filaggrin gene (*FLG*) mutations, as a result of degradation of tight junction proteins and corneodesmosomes (7). Accordingly, *FLG* null mutations were associated with a higher prevalence of nickel contact sensitization in German adults and in Danish adults without pierced ears (8, 9). Patch test

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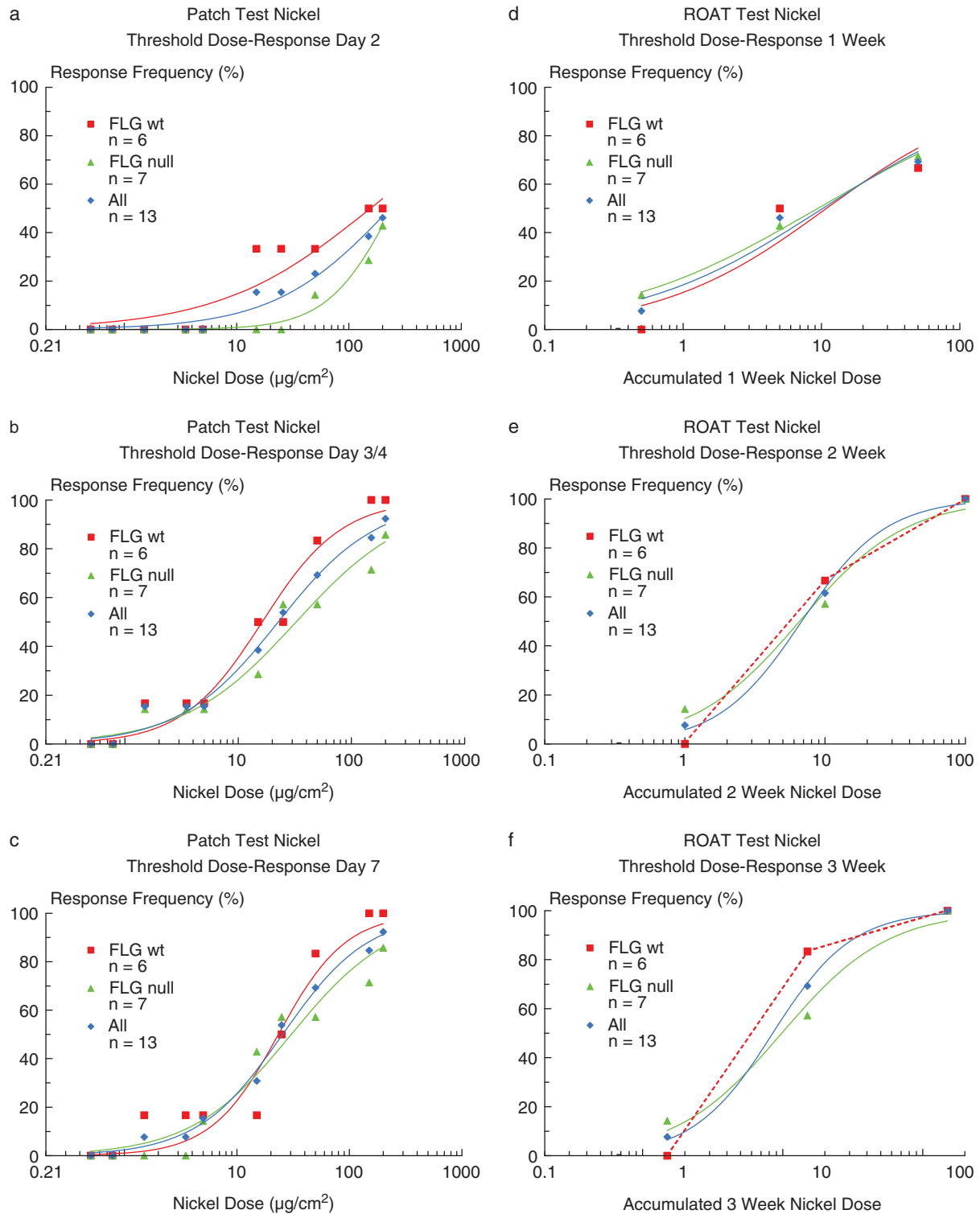


Fig. 1. Observed response frequencies and estimated logistic threshold dose–response curves from patch testing D1, D3 or D4, and D7 (a–c), and repeated open application test (ROAT) week 1–3 (d–f), stratified by filaggrin genotype (*FLG*). wt, non-mutation carriers; null, mutation carriers. The dose–response curves from ROAT week 2 and 3 (e, f) could not be defined for wild-type individuals, and the dose–response association is indicated by a dotted line connecting the observed frequencies.

Table 1. Patch test statistics

	<i>FLG</i> genotype	n	$\alpha \pm \text{SE}$	$\beta \pm \text{SE}$	Hypothesis tests ^a
Day 2	Wild type	6	-3.2480 ± 1.5503	0.6434 ± 0.3180	$\beta_1 = \beta_2: \chi^2(1) = 1.00, p = 0.32$
	Null	7	-8.2122 ± 4.0904	1.4953 ± 0.7926	
	All	13	-4.2081 ± 1.5831 ($\delta = -0.8429 \pm 1.0911$)	0.8657 ± 0.3088	
Day 3/4	Wild type	6	-3.4461 ± 1.4626	1.2355 ± 0.4312	$\beta_1 = \beta_2: \chi^2(1) = 0.49, p = 0.49$
	Null	7	-3.0310 ± 1.2177	0.8708 ± 0.2966	
	All	13	-2.8062 ± 0.9952 ($\delta = -0.7253 \pm 0.9906$)	1.0162 ± 0.2467	
Day 7	Wild type	6	-4.5498 ± 1.9151	1.4438 ± 0.5398	$\beta_1 = \beta_2: \chi^2(1) = 0.57, p = 0.45$
	Null	7	-3.3135 ± 1.2446	0.9702 ± 0.3219	
	All	13	-3.5268 ± 1.1306 ($\delta = -0.3148 \pm 0.9818$)	1.1340 ± 0.2774	

SE, standard error.

^aSubscripts 1 and 2 refer to wild type and null, respectively.**Table 2.** Repeated open application test statistics

	<i>FLG</i> genotype	n	$\alpha \pm \text{SE}$	$\beta \pm \text{SE}$	Hypothesis tests ^a
Week 1	Wild type	6	-1.7116 ± 0.9765	0.7169 ± 0.3104	$\beta_1 = \beta_2: \chi^2(1) = 0.12, p = 0.73$
	Null	7	-1.2971 ± 0.8488	0.5787 ± 0.2482	
	All	13	-1.5571 ± 0.8283 ($\delta = 0.1359 \pm 1.0116$)	0.6391 ± 0.1943	
Week 2	Wild type	6	b	b	$\delta = 0: \chi^2(1) = 0.00, p = 0.99$
	Null	7	-2.1587 ± 1.1468	1.1476 ± 0.4446	
	All	13	-2.7939 ± 1.2001 ($\delta = 0.0119 \pm 1.0992$)	1.4536 ± 0.4451	
Week 3	Wild type	6	b	b	$\delta = 0: \chi^2(1) = 0.27, p = 0.61$
	Null	7	-2.6240 ± 1.2916	1.1476 ± 0.4446	
	All	13	-3.0347 ± 1.2930 ($\delta = -0.5969 \pm 1.1573$)	1.5577 ± 0.4684	

SE, standard error.

^aSubscripts 1 and 2 refer to wild type and null, respectively.^bThe logistic threshold dose-response curves could not be defined. However, the comparison statistics were performed.

reactivity data have even indicated that *FLG* mutation carriers might have a lower elicitation threshold than non-mutation carriers, as a tendency for there to be stronger patch test reactions to nickel was observed (9). Here, we evaluated the nickel elicitation threshold in *FLG* mutation carriers and controls by performing a dose–response study including patch testing and repeated open application tests (ROATs).

Materials and Methods

Participants, patch testing, and ROAT

Between October 2011 and May 2012, 13 women aged 22–69 years were contacted and recruited from the patient database at the department of Dermato-Allergology, Copenhagen University Hospital Gentofte, Denmark. The study was conducted in accordance with the principles of the Declaration of Helsinki. It

was approved by the Ethics Committee of Copenhagen (H-1-2010-062), and informed written consent was obtained from study participants. Genotyping for the R501X, 2282del4 and R2447X *FLG* mutations was performed. A total of seven heterozygous mutation and six non-mutation carriers were enrolled, patch tested and subjected to ROAT with nickel sulfate dilutions ranging from 0 to 200 $\mu\text{g Ni/cm}^2$, as previously performed by Fischer et al. (10). Three of the mutation carriers and two of the non-mutation carriers had previously been diagnosed with atopic dermatitis. None had active dermatitis at study initiation, but all had previously suffered from dermatitis. Apart from the difference in *FLG* genotype, the participants seemed to be fairly comparable. Thus, 2 of the participants, 1 mutation carrier and 1 non-mutation carrier, had their first diagnosis of nickel sensitization made on the basis of patch testing performed in secondary dermatology clinics in Denmark prior to referral. For the participants who were initially diagnosed

with nickel sensitization in the Gentofte University Clinic, 2 of 6 mutation carriers and 3 of 5 non-mutation carriers had 2+ reactions on readings performed on day 3. The remaining participants had only 1+ reactions. The average times after the initial diagnosis of nickel sensitization were, respectively, 175 and 180 months for mutation and non-mutation carriers. In the present study, 1 mutation carrier and 1 non-mutation carrier had their patch tests read on day 4, whereas the rest of the participants were read on day 3.

Logistic threshold dose–response analyses

The probability of a positive skin response following nickel contact was presumed to be 0 if the applied dose was below the threshold dose and 1 if the applied dose was above the threshold dose. The logistic dose–response threshold model describes the distribution of the threshold dose, by defining the probability of a positive reaction, $P(x)$, at the dose x : $P(x) = 1/(1 + \exp[-\alpha - \beta \ln(x)])$. Graphically, $P(x)$, as a function of $\ln(x)$, is a sigmoidal proceeding from 0 to 1 if β is positive. It was tested whether the dose–response curves for *FLG* mutation and non-mutation carriers, respectively, were parallel (equal β values) and/or were coincident in proportion to the x -axis (equal α values). In practice, one group was described with the parameters (α, β) , and the other was described with $(\alpha + \delta, \beta)$. Hence, the null hypothesis is that $\delta = 0$. If the null hypothesis is accepted, the dose–response association can be explained by a common curve for all individuals irrespective of their *FLG* genotype.

Results

No participants reacted with visible skin changes in the control tests consisting of 90% H₂O and 10% EtOH, whereas all patients reacted to the highest nickel dose used for patch testing (200 µg Ni/cm²) and to the accumulated dose of 100 µg Ni/cm² in the ROAT. As seen from the observed and calculated dose–response curves shown in Fig. 1 and the logistic threshold dose–response analyses (Tables 1 and 2), no statistically significant differences were found in the dose–response relationship between *FLG* mutation and non-mutation carriers. The observed

response frequencies were similar in both the patch test (Fig. 1a–c) and the ROAT (Fig. 1d–f). The estimated logistic threshold dose–response curves were therefore nearly coincident. Thus, the dose–response associations can be explained by one common curve for all individuals irrespective of their *FLG* genotype on the given test day.

Discussion

Although *FLG* null mutations have been positively associated with nickel patch test reactivity and, especially, allergic nickel dermatitis in general population studies (8, 9), the threshold for experimental nickel contact elicitation appeared to be independent of *FLG* genotype in a small patient cohort from a tertiary clinic in Denmark. Recently, we showed that human epidermal filaggrin binds nickel strongly (5), which supports the idea that filaggrin does indeed contribute to the nickel-binding capacity of the epidermis and thereby ultimately could help to reduce nickel penetration through the stratum corneum and the induction of contact sensitization. The low number of patients included in this study could explain the discrepancy between the present and the previous studies. Importantly, it is well established that, among nickel-allergic dermatitis patients, the elicitation threshold may differ by 250-fold (11). Thus, the induction dose could, by coincidence, vary widely among patients in the two study groups, affecting the elicitation threshold to a much higher degree than inherited epidermal filaggrin levels (12). Notably, exogenous stressors such as low environmental humidity can affect epidermal filaggrin levels and thereby reduce the effect of inherited filaggrin deficiency (13). It may be that the inclusion only of individuals who had 3+ reactions in recent, diagnostic patch testing would have provided a better study group with which to reveal small elicitation threshold differences in the range potentially affected by *FLG* genotype. It would definitely be interesting to monitor a study group including individuals homozygous for *FLG* null mutations, owing to their complete lack of filaggrin and its metabolites in the stratum corneum. In conclusion, the experimental elicitation threshold level for nickel appears to be independent of single-allele *FLG* null mutation status.

References

- 1 Wells G C. Effects of nickel on the skin. *Br J Dermatol* 1956; **68**: 237–242.
- 2 Fullerton A, Hoelgaard A. Binding of nickel to human epidermis in vitro. *Br J Dermatol* 1988; **119**: 675–682.
- 3 Sandilands A, Sutherland C, Irvine A D, McLean W H I. Filaggrin in the frontline: role in skin barrier function and disease. *J Cell Sci* 2009; **122**: 1285–1294.
- 4 Rawlings A V, Scott I R, Harding C R, Bowser P A. Stratum corneum moisturization at the molecular level. *J Invest Dermatol* 1994; **103**: 731–741.
- 5 Ross-Hansen K, Østergaard O, Tanassi J T, Thyssen J P, Johansen J D, Menné T, Heegaard N H H. Filaggrin is a

- predominant member of the denaturation-resistant nickel binding proteome of human epidermis. *J Invest Dermatol* 2013; doi: 10.1038/jid.2013.445 [Epub ahead of print].
- 6 Thyssen J P, Carlsen B C, Menné T. Nickel sensitization, hand eczema, and loss-of-function mutations in the filaggrin gene. *Dermatitis* 2008; **19**: 303–307.
 - 7 Gruber R, Elias P M, Crumrine D et al. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. *Am J Pathol* 2011; **178**: 2252–2263.
 - 8 Novak N, Baurecht H, Schafer T et al. Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. *J Invest Dermatol* 2007; **128**: 1430–1435.
 - 9 Ross-Hansen K, Menné T, Johansen J D et al. Nickel reactivity and filaggrin null mutations – evaluation of the filaggrin bypass theory in a general population. *Contact Dermatitis* 2011; **64**: 24–31.
 - 10 Fischer I A, Johansen J D, Menné T. Nickel allergy: relationship between patch test and repeated open application test. *Br J Dermatol* 2007; **157**: 723–729.
 - 11 Emmet E A, Risby T H, Jiang L, Ng S K, Feinman S. Allergic contact dermatitis to nickel: bioavailability from consumer products and provocation threshold. *J Am Acad Dermatol* 1988; **19**: 314–322.
 - 12 Friedmann P S. The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. *Br J Dermatol* 2007; **157**: 1093–1102.
 - 13 Bouwstra J A, Wouter H, Groenink W, Kempenaar J A, Romeijn S G, Ponc M. Water distribution and natural moisturizer factor content in human skin equivalents are regulated by environmental relative humidity. *J Invest Dermatol* 2008; **128**: 378–388.