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Nickel penetration into stratum corneum in *FLG* null carriers—A human experimental study

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Abstract

Background: The filaggrin gene (*FLG*) plays a role in skin diseases, with the skin barrier function being impaired in *FLG* null carriers. The role of *FLG* status in relation to nickel penetration into the skin remains unclear.

Objectives: To elucidate the association between *FLG* status and nickel penetration into stratum corneum (SC) in individuals without self-reported history of nickel allergy.

Methods: Forty participants (23 *FLG* wt and 17 *FLG* null) were exposed to a nickel solution (80 μg/cm^2) which was applied onto 2 × 2 cm on their left forearm. After 4 h, the area was tape-stripped with 10 consecutive tapes. Nickel in each tape was quantified using inductively coupled plasma mass spectrometry.

Results: The average recovered nickel dose was 35%–48%. A tendency towards lower recovery was seen in *FLG* null carriers compared to *FLG* wt carriers, and lower recovery in those with history of skin and/or respiratory symptoms compared to those without such history. This was however not statistically significant.

Conclusion: *FLG* null carriers had less nickel recovered by tape strips compared with *FLG* wt carriers and, compared with individuals without a history of skin and/or respiratory symptoms, indicating higher nickel penetration into SC for *FLG* null carriers, but further studies are needed.

KEYWORDS
dermal exposure, *FLG* status, genetic susceptibility, human exposure study, nickel penetration, respiratory symptoms, skin symptoms

1 | INTRODUCTION

Nickel exposure in the general population and different occupational groups has been studied in detail by several authors.[1–6] The link to contact allergy is also well-established, with prevalence numbers in the range of 8%–18% in general populations and 12%–26% in dermatitis patients across Europe and North America[7,8] making nickel allergy the most common contact allergy in these regions.

How quickly nickel penetrates the skin in humans is not equally well studied. The term penetration in this article context is defined as nickel present in the stratum corneum (SC), and should not be considered as skin absorption, which corresponds to nickel becoming systemically

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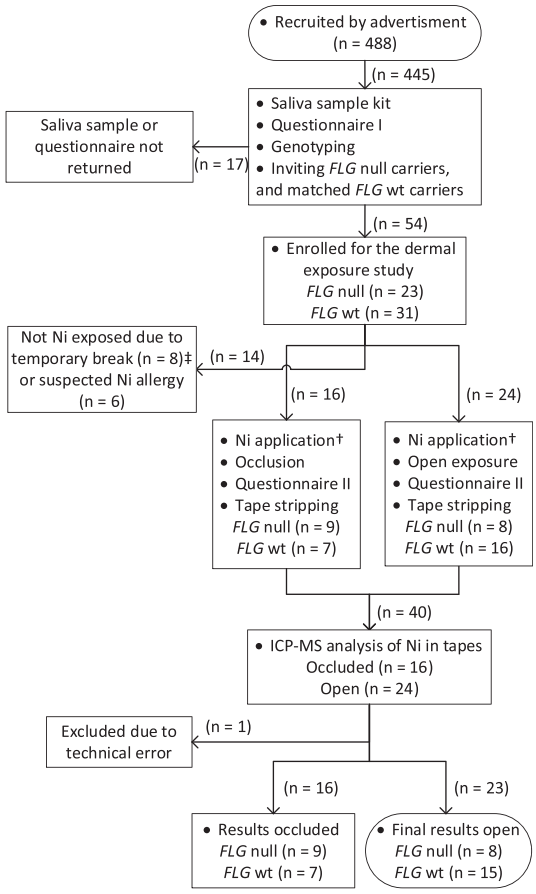
available by penetration through all the layers of skin. Nickel-allergic patients have reported dermatitis reactions within 30 min after skin contact with shiny metallic items.[9] To understand if short and repeated contact with nickel-releasing items may result in allergic contact dermatitis and nickel penetration into SC, two experimental studies were performed in nickel-allergic persons and controls.[10,11] They showed that nickel exposure from short contact (3 × 10 min) with nickel discs may result in dermatitis[11] and that nickel could be detected in the SC 72 h post-exposure, using the tape-stripping method.[10]

In vitro methods using diffusion cells for assessment of percutaneous absorption, measure penetration of chemicals into the skin and their permeation into a receptor fluid. This methodology has been used with ex vivo human or piglet skin to study permeation of aqueous nickel chloride, nickel sulphate,[12–14] and pure nickel dissolved in HNO_3.[15] Collectively, the studies show that nickel in different forms may penetrate into skin, even after short duration of exposure.

It is well-known that the filaggrin gene (*FLG*) plays a role in atopic dermatitis and ichtyosis vulgaris, and that the skin barrier function is impaired in *FLG* null carriers.[16–19] In a recent publication, studying the penetration of oxybenzone (UV-filter), pyrene (PAH), and pyrimethanil (fungicide), we have shown that *FLG* status affects the skin absorption and that *FLG* null carriers had the shortest lag-time and also higher levels of metabolites of PAH and pyrimethanil, and of oxybenzone in urine.[20] Studies of an association between *FLG* mutations and contact allergy, including nickel allergy, and nickel penetration into skin, however, have shown conflicting results.[21–25] Thus, the role of *FLG* status in relation to nickel penetration into the skin remains unclear.

The motivation for the present study was to elucidate the association between *FLG* status and nickel penetration into SC by performing an exposure study with well characterized individuals, with and without *FLG* null mutations. The motivation was also to study the association between nickel penetration and self-reported history of skin or respiratory symptoms.



n, number of individuals; Ni, nickel
† Lower left arm, washing before Ni application and after tape stripping, 10 strips per area
‡ Owing to visible skin reactions by occlusion of applied Ni

FIGURE 1 Flow chart of participants. Results of occluded exposure are presented in Figures S1 and S2.

2 | MATERIALS AND METHODS

2.1 | Study population, recruitment, and genotyping

The Regional Ethics Committee in Lund, Sweden approved the study (ID no. 2017/940). The study group has been described previously.[20] In short, individuals were recruited through advertising for non-smokers above 18 years of age. Four hundred eighty-eight persons were interested in the study, and after receiving information, 455 persons gave oral and written informed consent to participate in the study. DNA genotyping was performed by sending out a saliva sampling kit (Oragene DNA OG-500-kit; DNA Genotek) to the participants, together with a questionnaire. The questionnaire contained questions on, for example, occupation, snuff and/or smoking habits, and symptoms suggestive of nickel allergy. In total, the laboratory at the Division of Occupational and Environmental Medicine at Lund University, Sweden received 432 saliva kits and questionnaires for analysis.

DNA from saliva samples was extracted according to the manufacturers instruction, using the prepIT·LP2 extraction kit (DNA GenoTek). The single-nucleotide polymorphisms R501X (rs61816761), R2447X (rs138726443), S3247X (rs150597413), and the deletion mutation 2282del4 (rs41370446), representing the most common null mutations in northern Europe,[26] were determined using the TaqMan assay. Primers and probes used for analysis were bought from Thermo Fisher Scientific. Samples were analysed using a real-time polymerase chain reaction machine (7900HT; Applied Biosystems).

Based on the result of genotyping, 54 individuals (23 *FLG* null and 31 *FLG* wt carriers) were invited to take part in the nickel exposure study, which was performed simultaneously with exposure to oxybenzone, pyrene, and pyrimethanil.[20] All accepted the invitation and gave oral and written consent. At a later stage, some were not exposed to nickel ($n = 14$), owing to temporary break in nickel exposures (see below); ultimately, 40 individuals (17 *FLG* null and 23 *FLG* wt carriers) participated (Figure 1).

2.2 | Nickel exposure and tape stripping

A nickel containing solution was prepared from a stock solution of pure nickel dissolved in HNO_3 (10 000 μg/ml; SpectraScan; Teknolab). A final concentration of 4000 μg Ni/ml was prepared in 1% HNO_3 and 95% ethanol (1:1 by volume), which corresponds to a skin dose of 80 μg/cm^2 when applying 80 μl to a 2 × 2 cm surface area. This concentration is well below the concentration used in diagnostic patch testing for contact allergy (~300 μg nickel/cm^2) and that is considered safe.[27]

Before starting the exposure, the participants cleaned the lower left arm with soap and water. Thereafter a registered nurse rinsed the area with 1% HNO_3, not harmful to the skin,[5] followed by thorough rinsing with deionized water to ensure that no nickel was present on the skin. The nurse spread nickel solution evenly over a pre-marked (2 × 2 cm) area on the volar side of the lower left arm using the tip of a pipette. The solution was then allowed to dry. *FLG* null and *FLG* wt carriers were exposed at random order.

2.2.1 | Exposure with occlusion

After applying the nickel solution, the area was occluded using a disposable weighing boat of plastic, which was taped onto the arm using Mefix tape (Mölnlycke Health Care, Göteborg, Sweden). The purpose was to protect the exposed area from abrasion, without applying pressure by the occlusion. The occlusion was removed after 4 h, and the area was tape stripped (see below). Following exposure of the first 16 participants, three of them contacted us due to a visible skin reaction at or around the area where the nickel solution had been applied, and under the edge of the weighing boat, indicating nickel solution outside the test area (see Figure S1). The study was paused to evaluate the reactions (spotty erythema, some also with papules, all cleared within a few days), and to assess the concentration of nickel in the test solution. The three participants were offered referral to a dermatology clinic for patch testing to diagnose possible contact allergy to nickel, however, none of them accepted the offer.

2.2.2 | Open exposure

After assessment of the nickel solution, which was accurate, we resumed the nickel exposure study, but without occluding the nickel-exposed area. Except for one participant (diffuse skin reaction, declined referral to a dermatology clinic), no further reactions were reported by the subsequent 23 participants. For all four participants reporting symptoms, two were *FLG* null and two *FLG* wt carriers, and since all declined referral for patch testing, the nature of the reactions cannot be determined.

2.2.3 | Tape stripping

After 4 h of nickel exposure, the area was tape stripped with 10 consecutive D-Squame tapes (3.8 cm^2; D-Squame). The operator wore disposable gloves during tape stripping, and changed gloves between each participant. Each tape was applied using tweezers, followed by light pressure by hand for 5 s and was then removed from skin using the tweezers again. Each tape was then placed with the sticky side inwards in a microtube (2 ml polypropylene; VWR International), for transportation to the laboratory at Karolinska Institutet, Sweden for extraction. Then, the participants were asked to wash their arms with soap and water. Three blank samples were prepared each day, by taking unused tapes and directly place them in microtubes.

2.3 | Chemical analysis of nickel in tapes

The tapes were extracted following the procedure reported in a previous study.[10] In short, 2 ml of 67% HNO_3 (Normatom; VWR) was added to each microtube containing a tape, and it was left for 72 h in a ventilated hood. At this time point 150 μl of the extract was taken out of the sample and added to tubes (12 ml polypropylene, acid cleaned; Sarstedt, Nümbrecht, Germany) containing 9.85 ml of 2% HNO_3 acid (prepared from 67% HNO_3 and MilliQ-water Millipore; 18.2 MΩ/cm) and 5 ppb indium as an internal standard. Laboratory blanks were prepared for each extraction by taking 150 ml of 67% HNO_3 and added to 12 ml tubes as described above. The internal standard was prepared from a stock solution of 1000 μg/ml (SpectraScan; Teknolab). An 8-point calibration curve (0, 0.1, 1, 5, 10, 50, 100, and 500 μg/L) was prepared for inductively coupled plasma mass spectrometry (ICP-MS; iCAP Q; Thermo Fisher Scientific) analysis, using a 1000 μg/ml Ni-stock (SpectraScan; Teknolab). The limit of detection for the ICP-MS analysis was set to 0.02 μg/L using three times the standard deviation of laboratory blank samples.

2.4 | Questionnaires

Questionnaire I, including a question on symptoms related to metal exposure ('Have you ever had an itchy rash or eczema [redness, blisters, or scaling] from metal items?'), was distributed together with the saliva sample kit during the recruitment phase. The purpose of asking about symptoms from metal exposure was to identify and avoid engaging individuals who might be at risk of developing strong skin reactions by the experimental nickel exposure.

To evaluate background information on the history of skin and respiratory symptoms, respectively, we used Questionnaire II, based on the questions and response alternatives in the Nordic Occupational Skin Questionnaire 2002.[28] For the specific questions used, see Table S1.

2.5 | Statistics

To evaluate nickel penetration into the skin, we sorted the participants into the following six groups for comparison: *FLG* null versus *FLG* wt, skin symptoms versus no skin symptoms (based on answers

from questions D1, D2, and S5 in Table S1), respiratory symptoms versus no respiratory symptoms (based on answers to questions A1, A2, A3, and A4 in Table S1). *FLG* null was defined as having at least one mutation in R501X, R2447X, S3247X or the deletion mutation 2282del4. Skin symptoms and respiratory symptoms, respectively, were defined as at least one affirmative answer to questions regarding skin symptoms or respiratory symptoms (Table S1).

For both the descriptive statistics analyses and the group comparison analyses we subdivided the tape strips into three groups: Tapes 1–10, Tapes 1–2, and Tapes 3–10. The first group (Tapes 1–10) was used to evaluate the total recovered dose of nickel in comparison to the applied dose. According to the European Food and Safety Authority Guidance on dermal absorption[29] the two outermost tapes, when performing tape stripping in vivo, should be considered as a surface dose, not being available for absorption due to desquamation. Therefore, in the statistical analysis, we used recovered nickel in Tapes 1–2 as an indicator of a surface dose, as opposed to recovered nickel in Tapes 3–10 as an indicator of a penetrated dose.

For comparison of all six original groups with regards to recovery of applied dose in Tapes 1–10, we used the Kruskal–Wallis test and Dunn's multiple comparison test. For all other comparisons between two groups, we used the Mann–Whitney *U* test and considered a $p \leq 0.05$ as significant. All analyses and figures were made using GraphPad Prism version 8.3.0.

Overlap between participants related to history of skin and/or respiratory symptoms was visualized as an area-proportional Euler diagram (also mentioned Venn diagram) using circles (eulerAPE v3, http://www.eulerdiagrams.org/eulerAPE/).[30]

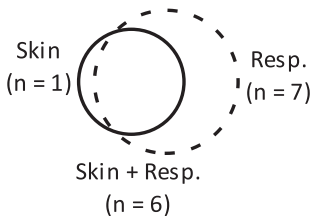


FIGURE 2 Area-proportional Euler diagram for the overlap between participants with self-reported history of skin and/or respiratory symptoms.

3 | RESULTS

During evaluation of the tape-strip results, we encountered systematically lower doses of nickel in tapes from study participants with occluded exposure. Likely explanations are that the nickel solution was not completely dry on skin before occlusion, allowing the solution to move outside the designated area for tape stripping; the weighing boats were also not assessed for possible deposition of nickel due to evaporation from exposed skin. The result of occlusion from the 16 participants is presented in Figure S2. The results from one participant without occlusion was omitted due to a technical error. This leaves in total 23 participants in the study with open exposure (Figure 1).

There was a large overlap among the participants with regards to self-reported history of skin and respiratory symptoms, as shown in Figure 2. Furthermore, an overlap was present between *FLG* null and respiratory symptoms ($n = 3$), and *FLG* null, respiratory symptoms and skin symptoms ($n = 2$).

In Figure 3, the distribution of the recovered dose of nickel in the individual tapes is displayed. As can be seen, the dose of nickel decreased in a dose dependent manner for all groups, with consistently lower doses recovered in the groups of *FLG* null, skin symptoms, and respiratory symptoms. It should be noted that in Tape 10, we could clearly detect a substantial amount of nickel (average 4 μg Ni), as compared to the limit of detection (0.003 μg), indicating that nickel had penetrated further into the SC.

The applied nickel dose (320 μg Ni on a 2 × 2 cm surface area) could be recovered in the range of 35%–48% (mean value) in the 10 tapes used in the study. The lowest recovery was found in the *FLG* null group and the highest in the group with no respiratory symptoms, however, the difference was non-significant (Table 1).

The group comparisons show that the median value is systematically lower for participants with *FLG* null compared to *FLG* wt, for those with skin symptoms as compared to no skin symptoms, and for respiratory symptoms as compared to no respiratory symptoms,

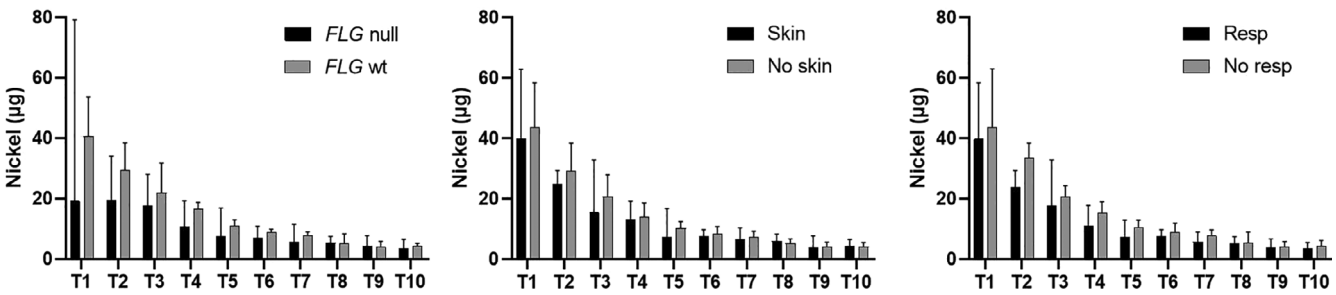


FIGURE 3 The distribution of recovered nickel (μg) presented as the median value and upper 95% CI for all 10 tapes. Data are presented group wise as: *FLG* null ($n = 8$) versus *FLG* wt ($n = 15$); skin symptoms (skin) ($n = 7$) versus no skin symptoms (no skin) ($n = 16$); and respiratory symptoms (resp) ($n = 13$) versus no respiratory symptoms (no resp) ($n = 10$).

TABLE 1 Descriptive statistics for the dose of recovered nickel (μg) in Tapes 1–10, 1–2, and 3–10 of the six different groups.

Group	n/N	Age mean (range)	Sex F/M	All tapes (1–10)			Surface dose (1–2)[a]			Penetrated dose (3–10)		
				Median	Range	p	Median	Range	p	Median	Range	p
FLG null	8/23	30.7 (22–52)	5/3	99.3	23.9–184.5	0.13	40.3	11.3–93.6	0.15	61.2	12.6–96.9	0.19
FLG wt	15/23	40.3 (21–74)	7/8	150.6	49.8–257.1		69.1	18.6–120.7		83.6	31.2–144.1	
Skin symptoms	7/23	36.9 (21–74)	5/2	130.0	93.7–184.5	0.72	61.0	38.7–87.7	0.72	69.0	43.2–102.1	0.62
No skin symptoms	16/23	37.1 (23–65)	7/9	142.2	23.0–257.1		66.8	11.3–120.7		75.3	12.6–144.1	
Resp. symptoms	13/23	33.1 (21–58)	7/6	112.5	49.8–257.1	0.65	64.4	18.6–120.7	0.69	64.4	31.2–144.1	0.64
No resp. symptoms	10/23	42.1 (24–74)	5/5	155.1	23.9–204.4		75.1	11.3–106.4		79.0	12.6–108.7	

Note: The total dose applied on skin was 320 μg Ni. The participants may be present in several groups depending on the answers in Questionnaire II.
Abbreviations: F, female; M, male; *n*, group size; *N*, total participants; Resp., respiratory.
[a]The first two tapes represent a skin dose that is not bioavailable due to desquamation.[29]

however, no statistically significant difference is present between the groups, as tested with the Mann-Whitney *U* test (Figure 4 and Table 1).

The median value of the penetrated dose (Tapes 3–10) is 61.2 μg Ni for *FLG* null and 83.6 μg Ni for *FLG* wt. The differences between corresponding values for those with and without skin symptoms, and respiratory symptoms, respectively, were smaller. None of the differences are statistically significant (Figures 2 and 4, Table 1).

4 | DISCUSSION

This study shows that nickel penetration into the SC occurs rapidly after skin exposure to nickel in solution. Already after 4 h, only 35%–48% of the applied dose could be recovered by tape stripping. Furthermore, we observed a tendency towards lower amounts of nickel in tapes from *FLG* null carriers compared to *FLG* wt carriers, and particularly for participants reporting a history of skin and/or respiratory symptoms, compared to no such symptoms, indicating more rapid penetration.

The application of a skin dose of nickel at 320 μg over a 4 cm^2 area (80 μg/cm^2) was chosen because it could represent an 8-h skin dose for metal workers' hands,[4] and because it is well below the dose used in diagnostic patch testing (nickel sulphate 5%, corresponding to 300 μg nickel/cm^2), which is considered safe from the perspective of patch-test sensitization.[27] The fact that we encountered some skin reactions after the nickel exposure is not surprising, considering that the prevalence of nickel allergy in general populations is 8%–18%.[7,8] We concluded that the reactions were either dermatitis owing to nickel allergy, or irritant reactions from the nickel solution. We aimed, by use of Questionnaire I, to avoid strong skin reactions and discomfort from the nickel exposure, which might occur in individuals with a high degree of sensitivity to nickel. To exclude proven nickel-allergic participants would have required patch testing, which was not feasible for the 455 subjects originally included for genotyping.

Various aspects of permeation of nickel through full-thickness skin have been studied using in vitro methods with diffusion cells employing nickel ions[15] or nickel powders.[31,32] Some key findings in the scientific literature of relevance for our study, are that the permeation rate is increased by occlusion per se,[33] and that the permeation rate of nickel particles and also the amount retained in the skin are increased when the skin surface has been experimentally abraded.[31,32] These findings are in concert with our experience indicating lower amounts of nickel recovered by tape stripping in the group with a history of skin and/or respiratory symptoms compared with no such symptoms, as well as in *FLG* null carriers that presumably have an impaired skin barrier function.

Fewer studies have been published concerning in vivo penetration of nickel in human skin.[10,14,33] A recent study used nickel in metallic form (nickel discs) as exposure medium.[10] That study showed that nickel penetrates SC already after a short exposure time (3 × 10 min), and that nickel could still be detected in the skin 72 h after exposure. Compared with the present study, where exposure

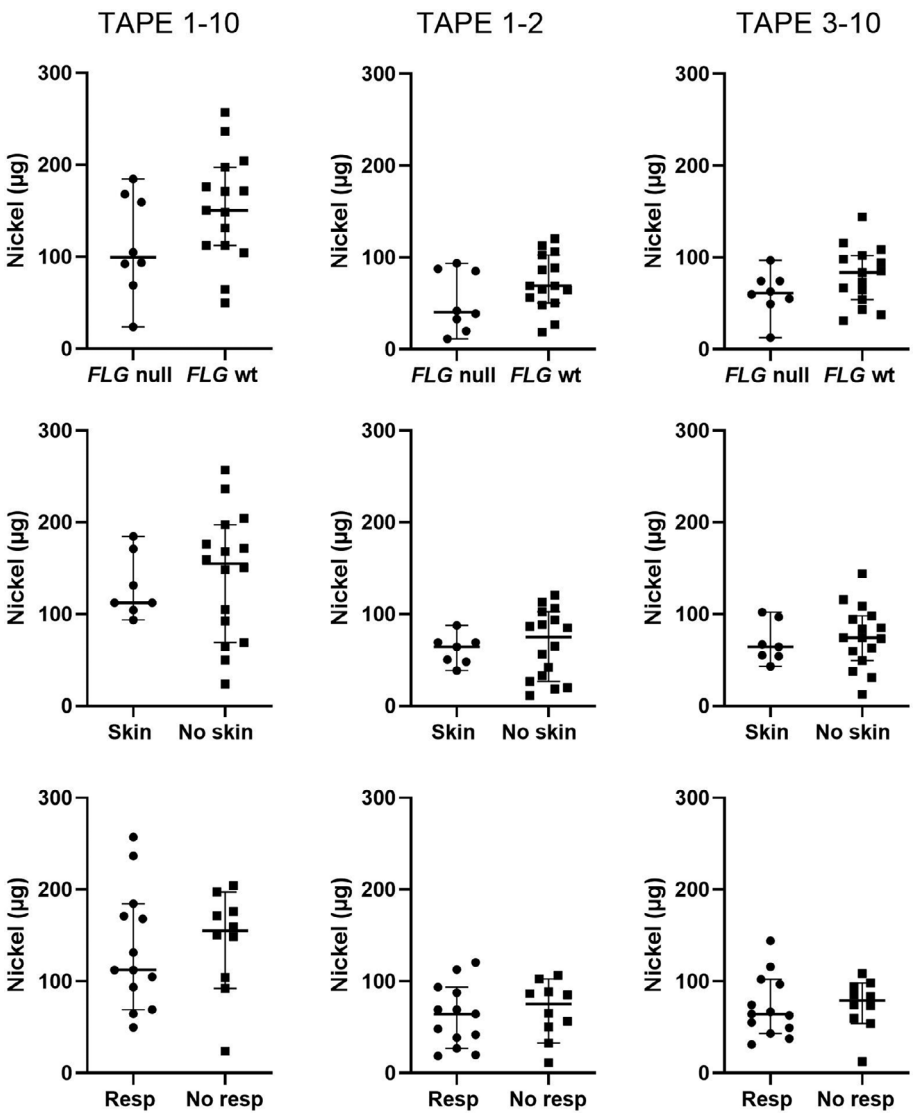


FIGURE 4 The sum of recovered Ni (µg) in Tapes 1–10, 1–2, and 3–10 presented as individual values, the median and 95% CI. Top row shows *FLG* null ($n = 8$) versus *FLG* wt ($n = 15$); middle row shows skin symptoms ($n = 7$) versus no skin symptoms ($n = 16$); and the bottom row shows respiratory symptoms ($n = 13$) versus no respiratory symptoms ($n = 10$).

was by nickel solution for 4 h, similar tendencies of rapid nickel penetration into the SC were seen. However, one important difference between the studies is that a larger proportion of the nickel dose was recovered in the SC (Tapes 3–10) than on the surface (Tapes 1–2) in the present study, compared with the study with short duration of exposure. Whilst Ahlström et al.[10] recovered approximately 70%–85% of the nickel in the two outermost tapes, we recovered 45% (mean value; range 28%–62%) of the nickel in the corresponding tapes. The difference could be attributed to exposure by metal discs, requiring nickel release before penetration, as compared to nickel ions being applied directly onto the skin, and to the difference in duration of exposure. Another explanation may be difference in pH, which is likely lower in the present study due to exposure with nickel solution in dilute HNO_3. pH has been shown to affect the permeation of rhodium and chromium in human ex vivo skin, where a reduction in pH from 6.5 to 4.5 significantly increased penetration.[34,35]

In the study using nickel salts, the pattern with a decreasing surface skin dose as a function of exposure time is clearly visible,[14] and likewise, the amount of nickel in SC as a function of the dose applied. The fact that the total recovered dose of nickel (Tapes 1–10) does not amount to more than 35%–48% of the applied dose in our study, might indicate that nickel has penetrated further into SC and the living epidermis. This assumption is supported by the fact that once reaching Tape 10, the recovered nickel dose is still high compared to both the blank samples and the LOD of the analytical method. Continued taping to reach deeper into the SC (20 tape strips or more to approach the glistening layer), might have recovered higher total doses of nickel but caused discomfort to the participants. But, as seen in the study by Hostýnek et al.,[14] this would probably still not have accounted for a total mass balance of nickel. The same finding of a constant level of nickel in skin between Tapes 10 and 20, has been reported in a study with humans exposed to nickel powder under occlusion.[33] A study using mass spectrometry for imaging nickel penetration in human ex vivo skin in Franz diffusion cells, indicates that nickel, after exposure for 24 h, was localized to the SC and upper living epidermis.[36] It has thus been shown with various methodologies that nickel penetrates skin quickly.

Studying the distribution profiles for nickel in Tapes 1–10 (Figure 3), we see the same pattern for all groups, with the highest amount of recovered nickel in Tape 1. *FLG* null carriers had the lowest recovered median dose in Tape 1 (19 μg Ni) compared to the median dose in Tape 1 (around 40 μg Ni) in the other groups. This is suggestive of a faster passage of nickel through the SC in the group with *FLG* null mutations and a history of skin and/or respiratory symptoms, known to have an impaired skin barrier function.[16–18] The fact that we do not find any statistically significant difference between the groups is likely due to the large variation between the individuals as displayed in Figure 4, where it is clearly shown that the 95% CI overlaps for all groups. Also, the large proportion of participants having both *FLG* null mutations and self-reported skin and/or respiratory symptoms may affect the possibility to evaluate their relative impact on nickel penetration into the SC. This, together with the relatively small sample size, likely, contributes to the fact that we cannot find statistically significant differences between the groups.

We are not aware of any study of nickel penetration in relation to *FLG* status suitable for comparison of results. The experimental study of nickel penetration using metal discs on skin[10] engaged nickel-allergic patients and non-nickel-allergic controls; only four participants were *FLG* null carriers. Several studies of *FLG* status and nickel allergy have been performed among patch tested dermatitis patients, occupational groups, and general populations. These studies have often shown conflicting results regarding associations between nickel allergy and *FLG* null mutations. Some studies have found associations,[23,24] while others negate such associations.[10,21,22]

Strengths of this experimental study are that it was performed in humans and that the applied skin dose of nickel is known, which allows for understanding of the penetration profile and calculation of the mass balance. Furthermore, the exposure time is longer than in a previous study[10] and is therefore relevant also for occupational settings with high nickel exposure. All participants were well characterized concerning self-reported history of skin and respiratory symptoms, as well as sex and age matched with regards to the *FLG* status.

The most obvious drawback of the study is the relatively few participants, compared to our initial intention. This was due to errors related to occlusion of the nickel-exposed area in the beginning of the study (unexpected skin reactions and leakage of the nickel solution). A larger study would probably have resulted in more concise groups and made the influence of the individual components smaller in relation to the individual values of nickel on tapes. Another drawback is the lack of measuring the amount of removed SC for each tape, which is known to vary between individuals.[37] Our study did not include individuals with suspected nickel allergy and, thus, does not provide new knowledge on possible associations between *FLG* status and nickel allergy.

5 | CONCLUSION

This study shows that the penetration profile of nickel in SC 4 h post-exposure follows the same pattern as previously described studies using nickel containing discs and different nickel salts. These results are also in concert with findings in in vitro studies. The results showed that *FLG* null carriers had non-significantly less nickel recovered by tape strips compared with *FLG* wt carriers and, compared with individuals without a history of skin and/or respiratory symptoms. This suggest that *FLG* null carriers may have a higher nickel penetration into SC, but further studies are needed. We consider it likely that *FLG* null carriers with nickel allergy, owing to their more efficient uptake through skin, may react with dermatitis at lower nickel exposure levels than *FLG* wt carriers.

AUTHOR CONTRIBUTIONS

Anneli Julander: Conceptualization (equal); data curation (lead); formal analysis (lead); methodology (equal); software (lead); validation (lead); writing – original draft (lead); writing – review and editing (equal). **Emelie Rietz Liljedahl:** Conceptualization (supporting); data curation (equal); formal analysis (supporting); investigation (equal); methodology (supporting); writing – review and editing (equal). **Helena Korres de Paula:** Investigation (equal); writing – review and editing (equal). **Eva Assarsson:** Investigation (equal); writing – review and editing (equal). **Malin Engfeldt:** Conceptualization (equal); methodology (equal); writing – review and editing (equal). **Margareta Littorin:** Investigation (equal); writing – review and editing (equal). **Christine Shobana Anto:** Formal analysis (equal); validation (equal); writing – review and editing (equal). **Carola Lidén:** Conceptualization (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Karin Broberg:** Conceptualization (lead); funding acquisition (lead); methodology (equal); project administration (lead); resources (lead); supervision (lead); validation (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Supporting information to:

Nickel penetration into stratum corneum in *FLG* null carriers – a human experimental study.

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Page 2. Table S1. Showing the questions from Questionnaire II, used for grouping participants into 4 different groups: “skin symptoms”; “no skin symptoms”; “respiratory symptoms” and “no respiratory symptoms”.

Page 3. Figure S1. Showing the difference in median nickel doses in tape 1 to 10 depending on *FLG* status and occlusion.

Page 4. Figure S2. Showing the weighing boat used for occlusion of exposure, and one case of skin reaction from occluded nickel exposure.

Table S1. Questionnaire II on self-reported symptoms in Phase 2, providing basis for the variables on skin symptoms and respiratory symptoms. Based on questions and response alternatives in the NOSQ-2002 questionnaire [32].

Source: NOSQ 2002 (sections: A, D, S)	Question	Response alternatives
A1.	Have you ever had an itchy rash that has been coming and going for at least 6 months, and at some time has affected skin creases?	☐ No ☐ Yes ☐ Don't know
A2.	Have you ever had "hay fever" or other symptoms of nasal allergy, e.g. from pollens or animals?	☐ No ☐ Yes ☐ Don't know
A3.	Have your eyes ever shown allergic symptoms, e.g. from pollens or animals?	☐ No ☐ Yes ☐ Don't know
A4.	Have you ever had asthma?	☐ No ☐ Yes ☐ Don't know
D1.	Have you ever had hand eczema?	☐ No ☐ Yes
D2.	Have you ever had eczema on your wrists or forearms (excluding fronts of elbows)?	☐ No ☐ Yes
S5.	Have you had childhood eczema?	☐ No ☐ Yes

Figure S1. A) Illustrates how the weighing boat was attached to the forearm, acting as occlusion for the nickel exposed area. B) Reaction after nickel exposure in one of three affected participants; at the area of application and tape stripping, and under the flat border of the weighing boat, indicating spread of the nickel solution beyond the area of application.

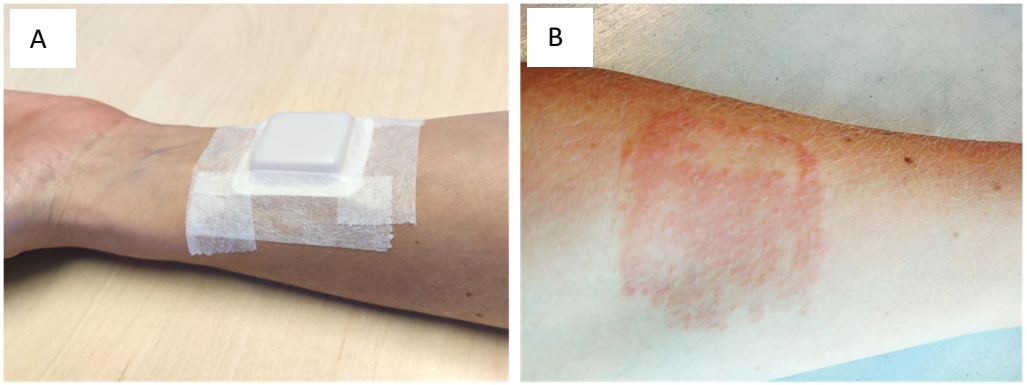

Photo taken 1-2 days after exposure
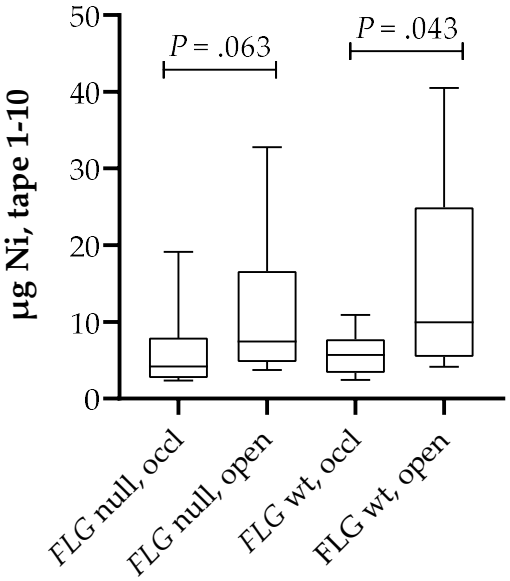
Figure S2. Systematically lower doses of nickel (µg) were recovered in the participants with occlusion compared to participants without occlusion irrespectively of the *FLG* status. Boxes represent the distribution of the mean value of nickel recovered in tape 1 to 10, with the whiskers showing min to max values. It is uncertain if the effect was due to higher absorption rate by occlusion, incomplete sampling due to spread beyond the site of application, or both.