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/cm²) which was applied onto 2 x 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 America7,8 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...
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. 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. They showed that nickel exposure from short contact (3 x 10 min) with nickel discs may result in dermatitis and that nickel could be detected in the SC 72 h post-exposure, using the tape-stripping method.

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, and pure nickel dissolved in HNO₃. 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 ichthyosis vulgaris, and that the skin barrier function is impaired in FLG null carriers. 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. Studies of an association between FLG mutations and contact allergy, including nickel allergy, and nickel penetration into skin, however, have shown conflicting results. 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.

MATERIALS AND METHODS

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. 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 manufacturer’s instructions, using the prepIT-LP2 extraction kit (DNA GenoTek). The single-nucleotide polymorphisms RS01X (rs61816761), R2447X (rs138726443), S3247X (rs150597413), and the deletion mutation 2282del4 (rs41370446), representing the most common null mutations in northern Europe, 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. 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).

FIGURE 1 Flow chart of participants. Results of occluded exposure are presented in Figures S1 and S2.
2.2 | Nickel exposure and tape stripping

A nickel containing solution was prepared from a stock solution of pure nickel dissolved in HNO₃ (10 000 μg/ml; SpectraScan; Teknolab). A final concentration of 4000 μg Ni/ml was prepared in 1% HNO₃ and 95% ethanol (1:1 by volume), which corresponds to a skin dose of 80 μg/cm² 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. Before starting the exposure, the participants cleaned the lower left arm with soap and water. Thereafter a registered nurse rinsed the area with 1% HNO₃, not harmful to the skin, 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 (Mölndlycke 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 cm²; 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. In short, 2 ml of 67% HNO₃ (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, Nürnberg, Germany) containing 9.85 ml of 2% HNO₃ acid (prepared from 67% HNO₃ 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% HNO₃ 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. 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 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 ≤ 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/).

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,
A recent study used nickel in human skin.

| Group          | n/N   | Age mean (range) | Sex F/M | Surface dose (3–10 μg/cm²) | All tapes (1–10) | Penetrated dose (3–10) | μg Ni | Range | p  |
|----------------|-------|------------------|---------|-----------------------------|------------------|------------------------|-------|-------|----|
| FLG null       | 8/23  | 30.7 (22–52)     | 5/3     | 23.9–184.5                  | 40.3–257.1       | 49.8–257.1             | 0.13 |       | 0.19|
| FLG wt         | 10/23 | 30.0 (21–74)     | 7/8     | 130.0–257.1                 | 18.6–120.7       | 31.2–154.1             | 0.15 | 0.19 | 0.19|
| Skin symptoms  | 7/23  | 120.0 (23–65)    | 5/2     | 61.0–120.7                  | 38.7–877.7       | 11.2–120.7             | 0.72 | 0.62 | 0.62|
| No skin symptoms | 16/23 | 142.2 (21–58)   | 8/7     | 120.7–204.4                 | 66.8–120.7       | 75.3–120.7             | 0.72 | 0.62 | 0.62|
| Resp. symptoms | 13/23 | 112.5 (21–58)   | 7/6     | 64.4–120.7                  | 49.8–257.1       | 64.4–18.6              | 0.69 | 0.64 | 0.64|
| No resp. symptoms | 10/23 | 42.1 (24–74)     | 5/5     | 75.1–204.4                  | 11.3–106.4       | 12.6–108.7             | 0.67 | 0.64 | 0.64|

**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.

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 to no such symptoms, as well as in FLG null carriers that presumably have an impaired skin barrier function.

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/cm² area (80 μg/cm²) was chosen because it could represent an 8-h skin dose for metal workers’ hands, and because it is well below the dose used in diagnostic patch testing (nickel sulphate 5%, corresponding to 300 μg nickel/cm²), which is considered safe from the perspective of patch-test sensitization.

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 or nickel powders. Some key findings in the scientific literature of relevance for our study, are that the permeation rate is increased by occlusion per se, 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. 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 to 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. A recent study used nickel in metallic form (nickel discs) as exposure medium. 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...
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. 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 HNO₃. 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, 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., 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.\textsuperscript{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\textsuperscript{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,\textsuperscript{23,24} while others negate such associations.\textsuperscript{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\textsuperscript{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.\textsuperscript{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 suggests 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).

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Margareta Littorin: Investigation (equal); writing – review and editing (equal).

Christine Shobana Anto: Formal analysis (equal); validation (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.

**REFERENCES**

1. Blisterbos J, Yazar K, Lidén C. Nickel on the Swedish market: follow-up 10 years after entry into force of the EU nickel directive. Contact Dermatitis. 2010;63(6):333-339.

2. Gawrkodger DJ, McLeod CW, Dobson K. Nickel skin levels in different occupations and an estimate of the threshold for reacting to a

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single open application of nickel in nickel-allergic subjects. Br J Dermatol. 2012;166(1):82-87.

3. Jensen P, Thyssen JP, Johansen JD, Skare L, Lidén C, Menne T. Occupational hand eczema caused by nickel and evaluated by quantitative exposure assessment. Contact Dermatitis. 2011;64(1):32-36.

4. Julander A, Skare L, Mulder M, Grändér M, Vahter M, Lidén C. Skin deposition of nickel, cobalt, and chromium in production of gas turbines and space propulsion components. Ann Occup Hyg. 2010;54(3):340-350.

5. Lidén C, Skare L, Nise G, Vahter M. Deposition of nickel, chromium and cobalt on the skin in some occupations—assessed by acid wipe sampling. Contact Dermatitis. 2008;58(6):347-354.

6. Ringborg E, Lidén C, Julander A. Nickel on the market: a baseline survey of articles in ‘prolonged contact’ with skin. Contact Dermatitis. 2016;75(2):77-81.

7. Ahlström MG, Thyssen JP, Menne T, Johansen JD. Prevalence of nickel allergy in Europe following the EU nickel directive—a review. Contact Dermatitis. 2017;77(4):193-200.

8. DeKoven JG, Warshaw EM, Zug KA, et al. North American contact dermatitis group patch test results: 2015-2016. Dermatitis. 2018;29(6):297-309.

9. Ahlström MG, Menne T, Thyssen JP, Johansen JD. Nickel allergy in a Danish population 25 years after the first nickel regulation. Contact Dermatitis. 2017;76(6):325-332.

10. Ahlström MG, Midander K, Menne T, et al. Nickel deposition and penetration into the stratum corneum after short metallic nickel contact: an experimental study. Contact Dermatitis. 2019;80(2):86-93.

11. Ahlström MG, Thyssen JP, Menne T, et al. Short contact with nickel causes allergic contact dermatitis: an experimental study. Br J Dermatol. 2018;179(5):1127-1134.

12. Fullerton A, Andersen JR, Hoelgaard A, Menne T. Permeation of nickel salts through human skin in vitro. Contact Dermatitis. 1986;15(3):173-177.

13. Hostynck J. Flux of nickel(II) salt versus a nickel(II) soap across human skin in vitro. Exog Dermatol. 2003;24(2):216-222.

14. Taneo H, Hostynck JJ, Mountford HS, Maibach HI. In vitro permeation of nickel salt through human stratum corneum. Acta Derm Venereol Suppl (Stockh). 2001;81(212):19-23.

15. Midander K, Schenk L, Julander A. A novel approach to monitor skin permeation of metals in vitro. Regul Toxicol Pharmacol. 2020;115:104693.

16. Brown SJ, Elias MS, Bradley M. Genetics in atopic dermatitis: historical perspective and future prospects. Acta Derm Venereol. 2020;100(12):adv00163.

17. Friedmann PS, Sanchez-Elsner T, Schmuck A. Genetic factors in susceptibility to contact sensitivity. Contact Dermatitis. 2015;72(5):263-274.

18. Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. N Engl J Med. 2011;365(14):1315-1327.

19. Weidinger S, Novak N. Atopic dermatitis. Lancet. 2016;387(10023):1109-1122.

20. Rietz-Liljedahl E, Johanson G, Korre de Paula H, et al. Filaggrin polymorphisms and the uptake of chemicals through the skin—a human experimental study. Environ Health Perspect. 2021;129(1):017002.

21. Carlsen BC, Johansen JD, Menne T, et al. Filaggrin null mutations and association with contact allergy and allergic contact dermatitis: results from a tertiary dermatology clinic. Contact Dermatitis. 2010;63(2):89-95.

22. Lagrellus M, Wahlgren CF, Bradley M, et al. Filaggrin gene mutations in relation to contact allergy and hand eczema in adolescence. Contact Dermatitis. 2020;82(3):147-152.

23. Novak N, Baurecht H, Schäfer T, et al. Loss-of-function mutations in the filaggrin gene and allergic contact sensitization to nickel. J Invest Dermatol. 2008;128(6):1430-1435.

24. Thyssen JP, Johansen JD, Linneberg A, et al. The association between null mutations in the filaggrin gene and contact sensitization to nickel and other chemicals in the general population. Br J Dermatol. 2010;162(6):1278-1285.

25. Thyssen JP, Linneberg A, Ross-Hansen K, et al. Filaggrin mutations are strongly associated with contact sensitization in individuals with dermatitis. Contact Dermatitis. 2013;68(5):273-276.

26. Thyssen JP, Blikke DD, Elias PM. Evidence that loss-of-function filaggrin gene mutations evolved in northern Europeans to favor intracutaneous vitamin D3 production. Evol Biol. 2014;41(3):388-396.

27. Johansen JD, Aalto-Korte K, Agner T, et al. European Society of Contact Dermatitis guideline for diagnostic patch testing – recommendations on best practice. Contact Dermatitis. 2015;73(4):195-221.

28. Susitaiw-P, Flyvholm MA, Meding B, et al. Nordic Occupational Skin Questionnaire (NOSQ-2002): a new tool for surveying occupational skin diseases and exposure. Contact Dermatitis. 2003;49(2):70-76.

29. EFSA. Guidance on dermal absorption. EFSA J. 2017;15(6):e04873.

30. Micallef L, Rodgers P. EulierAPE: drawing area-proportional 3-Venn diagrams using ellipses. PLoS One. 2014;9(7):e101717.

31. Crosera M, Adami G, Mauro M, Bovenzi M, Baracchini E, Larese FF. In vitro dermal penetration of nickel nanoparticles. Chemosphere. 2016;145:301-306.

32. Filon FL, D’Agostin F, Crosera M, Adami G, Bovenzi M, Maina G. In vitro absorption of metal powders through intact and damaged human skin. Toxicol In Vitro. 2009;23(4):574-579.

33. Hostynck JJ, Drehner F, Pelosi A, Anigbogu A, Maibaich HI. Human stratum corneum penetration by nickel. In vivo study of depth distribution after occlusive application of the metal as powder. Acta Derm Venereol Suppl (Stockh). 2001;212:5-10.

34. Janssen Van Rensburg S, Franken A, Du Plessis J, Du Plessis JL. The influence of pH on the in vitro permeation of rhodium through human skin. Toxicol Ind Health. 2017;33(6):487-494.

35. Larese Filon F, D’Agostin F, Crosera M, Adami G, Bovenzi M, Maina G. In vitro percutaneous absorption of chromium powder and the effect of skin cleanser. Toxicol In Vitro. 2008;22(6):1562-1567.

36. Malmberg P, Guttenberg T, Ericson MB, Hagvall L. Imaging mass spectrometry for novel insights into contact allergy—a proof-of-concept study on nickel. Contact Dermatitis. 2018;78(2):109-116.

37. Clausen M-L, Slotved HC, Krogfelt KA, Agner T. Tape stripping technique for stratum Corneum protein analysis. Sci Rep. 2016;6(1):19918.

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