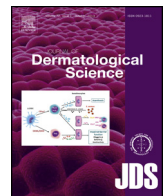




Contents lists available at ScienceDirect

Journal of Dermatological Science

journal homepage: www.jdsjournal.com



High frequencies of positive nickel/cobalt patch tests and high sweat nickel concentration in patients with intrinsic atopic dermatitis

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ARTICLE INFO

Article history:

Received 4 June 2013

Received in revised form 29 July 2013

Accepted 29 July 2013

Keywords:

Metal patch test

Filaggrin (FLG)

Barrier impairment

Extrinsic

Toll-like receptor (TLR)

Piercing

ABSTRACT

Background: Atopic dermatitis (AD) is classified into extrinsic AD with high serum IgE levels and impaired barrier, and intrinsic AD with low serum IgE levels and unimpaired barrier. Intrinsic AD has a lower frequency of *FLG* mutations and a higher frequency of circulating Th1 cells, implying that non-protein antigens, represented by metals, may be an exacerbation factor in intrinsic AD.

Objective: To investigate metal allergy in intrinsic AD.

Methods: Enrolled in this study were 86 Japanese AD patients seen in three university hospitals, consisting of 55 extrinsic and 31 intrinsic AD patients. Patch testing was performed, focusing on nickel, cobalt, and chrome, in parallel with other 14 metals. *FLG* mutations were analyzed in 49 patients (extrinsic, 29; intrinsic, 20). In 17 patients (extrinsic, 12; intrinsic, 5), sweat was collected from the forearms by exercise, and the concentration of nickel was fluorometrically measured.

Results: Nickel, cobalt, and chrome were the major positive metals. Intrinsic AD showed significantly higher percentages of positive reactions than extrinsic AD to nickel (intrinsic 41.9% vs extrinsic 16.4%, $P = 0.019$) and cobalt (38.7% vs 10.9%, $P = 0.005$). There was no significant difference between *FLG* mutation-bearing and non-bearing patients. The concentration of nickel was higher in the sweat of intrinsic AD than extrinsic AD patients (333.8 vs 89.4 ng/g, $P = 0.0005$) and inversely correlated with serum IgE levels.

Conclusions: Nickel and cobalt allergy may be involved in intrinsic AD. Given that the metals are excreted through sweat, intrinsic AD might be exaggerated by highly metal-containing sweat.

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1. Introduction

Atopic dermatitis (AD) can be divided into the extrinsic and intrinsic types [1,2]. While extrinsic AD shows high serum IgE levels with specific IgE to some allergens, intrinsic AD has low serum IgE levels without specific IgE. Since total serum IgE value correlates with the allergen-specific IgE status [3], the total IgE can be regarded as a clinically useful parameter to differentiate between the extrinsic and intrinsic types in both adults [4,5] and children [3]. The frequency of intrinsic AD is 10–45% of whole AD

patients [6], and it has more recently been reported to be 27% [7], 37% [3], 12% [8], and 22% [5]. The female preponderance is a feature of intrinsic AD [1,4,5,9,10].

Extrinsic AD is characterized by impaired barrier function [11]. Loss-of-function mutations in filaggrin gene (*FLG*) [12] represent a strong predisposing factor for AD, asthma and other allergies [13]. Perturbation of skin barrier function as a result of reduction or complete loss of *FLG* expression leads to enhanced percutaneous transfer of protein antigens [11,14]. Therefore, *FLG* mutations are closely associated with extrinsic AD [13,15] as well as ichthyosis vulgaris and palmar hyperlinearity. A recent finding also suggests that *FLG* deficiency results from perturbation of enzymatic processing from profilaggrin to filaggrin monomer [16]. On the other hand, intrinsic AD patients have normal barrier function [4,15] and virtually lack *FLG* mutations and palmar hyperlinearity [9,15].

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Recently, we have shown that the percentage of interferon (IFN)- γ -producing Th1 cells is significantly higher in the peripheral blood of intrinsic AD than extrinsic AD [15]. Protein antigen is known to induce Th2 responses [11,17], and therefore, the impaired barrier of extrinsic AD may allow protein allergens to penetrate the barrier and to evoke Th2 responses. In this scenario, Th1-inducing non-protein antigens, such as metals, might be causative for intrinsic AD [18]. In fact, high frequencies of positive patch test reactions to metals have been reported in AD patients [19–21]. Nickel (Ni), cobalt (Co) and chrome (Cr) are the three major metals, and a high frequency of positive patch test to at least one of them is higher in AD patients than in non-AD patients [21]. However, the intrinsic and extrinsic types were not separately analyzed in those studies. Metals are administered orally as food and may be excreted from sweat at high concentrations as well as urine [22]. This notion raises the possibility that the concentrations of metals are high in the sweat of intrinsic AD patients.

In this study, we performed a multicenter study of patch tests to metals, focusing on Ni, Co and Cr, in extrinsic and intrinsic AD patients. To better assess the patients' background, *FLG* mutations were investigated with other items. We also measured the concentration of Ni in the two groups of AD patients.

2. Materials and methods

2.1. Participants and background assessments

AD was diagnosed according to the criteria of Hanifin and Rajka [23] and divided into the intrinsic and extrinsic types. There are no uniform criteria to distinguish intrinsic AD from extrinsic AD, as IgE values less than 150, 200 or 220 kU/L have been used for an indication of intrinsic AD in the previous studies [2,4,6]. Since Japanese AD patients have high levels of IgE specific to house dust mites [17,24], IgE levels to *Dermatophagoides (D) pteronyssinus* (DP) and *D. farinae* (DF) were used for categorization of extrinsic and intrinsic AD as well as total IgE levels. Serum IgE levels specific to these mites were graded into 7 classes (class 0 to 6; Special Reference Laboratories, Tokyo, Japan). In this study, intrinsic AD was defined as serum IgE levels ≤ 200 kU/L or $200 < \text{IgE} \leq 400$ plus class 0 or 1 of IgE specific to DP or DF, and extrinsic AD was defined as $400 < \text{IgE}$ levels or $200 < \text{IgE} \leq 400$ plus class 2 or more of the specific IgE.

Enrolled in this study were 31 patients with intrinsic AD (aged 9–59 years; 10 men and 21 women) and 55 patients with extrinsic AD (aged 6–78 years; 22 men and 33 women) (Table 1). We first selected 31 intrinsic AD patients who visited the hospitals in

2009–2012, and as the control counterpart, 55 extrinsic AD patients were then randomly selected. The ages of the two groups were statistically comparable, but the female dominance was observed in intrinsic AD as well known [1,2,15]. The patients were seen as outpatients or inpatients in Departments of Dermatology, Hamamatsu University School of Medicine, University of Occupational and Environmental Health, and Kobe University.

The severity of AD was assessed by Severity Scoring of AD index (SCORAD) [25] and a 100 mm visual analog scale (VAS) was used for their itch. Serum CCL17/thymus and activation-regulated chemokine (TARC), strongly associated with severity of AD [26,27], was measured by ELISA (Special Reference Laboratories) in 26 extrinsic AD patients and 11 intrinsic AD patients.

2.2. Intracellular cytokine staining and flow cytometric analysis of peripheral blood mononuclear cells (PBMCs)

Peripheral blood mononuclear cells (PBMCs) were isolated by standard Ficoll-Paque method. Intracellular cytokines were stained according to the protocol of Cytostain (Immunotech, Marseille, France) [15]. Briefly, cells (2×10^6 cells/ml) were stained with PerCP-conjugated anti-CD8 monoclonal antibody (mAb), APC-conjugated anti-CD3 mAb (BD Biosciences), phycoerythrin-labeled anti-IL-4 or IL-5, and FITC-labeled anti-IFN- γ mAb (all from BD Biosciences). Fluorescence profiles were analyzed by flow cytometry in FACSCanto (BD Biosciences). The percentage of CD3⁺CD8⁺ cells bearing each cytokine was counted.

2.3. FLG mutation

Mutation in *FLG* has been identified as one of the most important factors of AD [12,26,28]. Blood was collected from 20 intrinsic and 29 extrinsic AD patients for detection of *FLG* mutation by polymerase chain reaction (PCR). Former reported mutations specific to Japanese were eight types (R501X, 3321delA, S1695X, Q1701X, S2554X, S2889X, S3296X and K4022X) [29,30]. Genotyping for the 8 types of *FLG* was performed using PCR and restriction enzyme digest analysis [31]. The analysis was approved by the ethical committee of Hamamatsu University School of Medicine. Patients gave written informed consent for the participation in this study, which followed the principles of the Declaration of Helsinki.

2.4. Patch testing with metals

A multicenter study was performed in the Departments of Dermatology of the three university hospitals. Patch testing with

Table 1
Patients' background in this study.

	Intrinsic AD	Extrinsic AD	
Patients	31 (10 men and 21 women)	55 (22 men and 33 women)	
Age (years, mean $\pm$ SD)	30.9 $\pm$ 11.5	28.9 $\pm$ 13.5	$P = 0.501$
Sex (male:female, %)	32.3%:67.7%	40.0%:60.0%	$P = 0.498$
IgE (kU L ⁻¹ , mean $\pm$ SD)	168.7 $\pm$ 124.3 ($n = 31$)	8829.5 $\pm$ 12508.0 ($n = 55$)	$P < 0.001$
LDH (IU L ⁻¹ , mean $\pm$ SD)	243.0 $\pm$ 143.5 ($n = 22$)	266.3 $\pm$ 118.7 ($n = 43$)	$P = 0.489$
Eosinophil (% mean $\pm$ SD)	7.0 $\pm$ 6.7 ($n = 25$)	9.57 $\pm$ 6.3 ($n = 42$)	$P = 0.115$
TARC (pg mL ⁻¹ , mean $\pm$ SD)	783.1 $\pm$ 826.1 ($n = 11$)	2223.1 $\pm$ 3596.1 ($n = 26$)	$P = 0.200$
Circulating Th1/Th2 cells (%) (% mean $\pm$ SD)			
IFN- γ	12.94 $\pm$ 4.62 ($n = 19$)	8.66 $\pm$ 6.51 ($n = 31$)	$P = 0.016$
IL-4	0.35 $\pm$ 0.15 ($n = 19$)	0.35 $\pm$ 0.23 ($n = 33$)	$P = 0.905$
IL-5	0.47 $\pm$ 0.23 ($n = 18$)	0.58 $\pm$ 0.26 ($n = 33$)	$P = 0.145$
VAS of pruritus (mean $\pm$ SD)	56.1 $\pm$ 31.2 ($n = 20$)	55.4 $\pm$ 22.7 ($n = 33$)	$P = 0.931$
SCORAD (mean $\pm$ SD)	36.0 $\pm$ 15.2 ($n = 20$)	40.2 $\pm$ 17.3 ($n = 34$)	$P = 0.369$
FLG mutation	5.0% (1/20:M0/6:F 1/14)	31.0% (9/29: M 6/11, F 3/18)	$P = 0.065^*$
Sweat sample collection	5 (3 men and 2 women)	12 (6 men and 6 women)	
Piercing history in female patients	40.0% (6/15) ^a	13.0% (3/23) ^a	$P = 0.115^*$

* Fischer's exact test.

^a In the piercing history-positive patients, 3 of 6 in intrinsic AD and none of 3 in extrinsic AD experienced contact dermatitis due to piercing metals.

metals was done in all 86 patients (31 intrinsic and 55 extrinsic AD patients) with three different patch test materials (PTMs), focusing on Ni, Co, and Cr as the major metals tested. Three different PTMs were used in each of the three hospitals. PTM-1, used in University of Occupational and Environmental Health, was obtained from Torii Pharmaceutical Co. Ltd., Tokyo, Japan, as Japanese Standard Metal Allergen Series, and other fourteen metals were also tested with PTM-1, including aluminum chloride, stannous chloride, ferric chloride, platinum chloride, palladium chloride, manganese chloride, indium trichloride, iridium tetrachloride, silver bromide, potassium dichromate, zinc chloride, gold trichloride, cupric sulfate, and mercuric chloride. PTM-2, used in Hamamatsu University School of Medicine, was purchased from Sato Pharmaceutical Co. Ltd., Tokyo, Japan. PTM-3, used in Kobe University, was provided by The Japanese Society for Dermatoallergology and Contact Dermatitis. The concentrations of Ni, Co and Cr were different among the three PTMs. PTM-1 is of liquid type, containing 5% Ni, 2% Co, and 0.5% Cr. PTM-2 is of plaster type, containing 34.8% Ni, 52.3% Co, and 31.2% Cr. PTM-3 is of petrolatum type, containing 2.5% Ni, 1.0% Co, and 0.5% Cr. The patch tests were applied to the upper back and occluded for 48 h. Reactions were assessed on day 2 and day 7 in most cases, with ± 1 day variations in some cases, according to the International Contact Dermatitis Research Group and scored as 1+, 2+, and 3+. A 1+, 2+ or 3+ reading was interpreted as a positive response.

2.5. Quantitative analysis of Ni in sweat

Sweat samples of 12 extrinsic AD patients and 5 intrinsic AD patients were collected. The forearms of the patients were washed clean, and the patients ran on the treadmill with his forearms wrapped with plastic bag for 10–15 min. The collected sweat was filtered with 0.45 μ m diameter membrane (Millex, Merck Millipore, Billerica, MA). The concentrations of Ni were measured fluorometrically with Inductively Coupled Plasma Atomic Emission Spectroscopy (Mitsubishi Chemical Analytec Co. Ltd., Tokyo, Japan).

2.6. Statistical analyses

Student's *t* test and Fisher's exact test were applied to determine calculating differences. *P*-value <0.05 was defined statistically significance.

3. Results

3.1. Patients' demographics

Patients' background is summarized in Table 1. The ages of the intrinsic and extrinsic groups were statistically comparable. The female preponderance was observed in intrinsic AD as reported previously [1,2,4,9,10,15], but the extrinsic AD patients in this study also consisted of a higher number of women. The extrinsic AD group tended to have high values of serum LDH, eosinophils counts, VAS of pruritus, SCORAD, and serum TARC, compared with the intrinsic AD group, without statistical significance. Both groups showed high frequencies of IL-4⁺ and IL-5⁺ T cells in the peripheral blood, but IFN- γ ⁺ T cells were higher in intrinsic AD than in extrinsic AD [15]. The higher levels of SCORAD and VAS in extrinsic than intrinsic AD have also been observed [4,8,32]. As to *FLG* mutation, 3.7% of normal Japanese individuals have *FLG* mutation [30], and extrinsic AD patients have higher frequency of *FLG* mutations than intrinsic AD patients [15]. In our patients, 9 of 29 extrinsic AD patients had one of the eight mutations common to Japanese AD patients, whereas only one of 20 intrinsic AD patients had a mutation of *FLG*. Piercing might bypass the epidermal barrier

and induce metal allergy, in particular Ni allergy [33]. Totally 70 patients were surveyed in their histories of piercing. Since only one male patient had a history of piercing, data from the female patients are shown in Table 1. Six of 15 female intrinsic AD patients had piercing histories, while 3 of 23 female extrinsic AD patients had piercing. Three of 6 female intrinsic patients experienced contact dermatitis to piercing metals, while none of 3 female extrinsic patients did so.

3.2. High frequencies of positive patch test reactions to Ni and Co in intrinsic AD than extrinsic AD patients

Patch testing to metals was performed with the three different materials, PTM-1 (liquid type), PTM-2 (plaster type), and PTM-3 (petrolatum type). First, we examined the positive reactions to 17 metals by using PTM-1 in 14 intrinsic and 21 extrinsic AD patients. Among 17 metals, high percentages of positive reactions were found with Ni (intrinsic 4/14, 28.6%; extrinsic, 6/21, 28.6%), Co (intrinsic 8/14, 57.1%; extrinsic, 2/21, 9.5%), and Cr (intrinsic 4/14, 28.6%; extrinsic, 4/21, 19.1%). However, there were low positive frequencies in manganese (intrinsic 2/14, 14.3%; extrinsic, 1/21, 4.8%), palladium (intrinsic 1/14, 7.1%; extrinsic, 1/21, 4.8%), zinc (intrinsic 1/14, 7.1%; extrinsic, 2/21, 9.5%), aluminum (0%), stannous (0%), ferric (0%), platinum (0%), indium (0%), iridium (0%), silver (0%), potassium (0%), gold (0%), cupric (0%), and mercuric (0%). Thus, Ni, Co, and Cr were the major metals that evoke positive patch test reactions.

We therefore focused on Ni, Co, and Cr as the metals to be tested by using the three different PTMs, which provided virtually similar results. However, there were considerable variations among the PTMs, presumably because of differences in metal concentrations and vehicle materials (Table 2). When analyzed collectively with the three PTMs, Ni, Co, and Cr exhibited positive reactions at 25.6%, 20.9%, and 16.3%, respectively, in the 86 AD patients.

We then analyzed the positive frequencies in the intrinsic and extrinsic types of AD. Intrinsic AD showed significantly higher percentages of positive reactions than extrinsic AD to Ni and Co (Table 3). The positivity to Co was also higher in intrinsic than extrinsic AD, but statistically not significant. The prevalence of metal allergy to one or more of the three metals was more than twice higher in intrinsic AD (61.3%) than extrinsic AD (25.5%).

We found the relationship between the stratification of IgE levels and the prevalence of positive metal patch tests (Fig. 1). In the IgE ≤ 100 group, the incidence of positive reactions to one or more of Ni, Co, and Cr was 63.6%, while the 400 < IgE group exhibited 25.0% positivity (*P* = 0.029). The group of 100 < serum IgE ≤ 400 (IU/L) consisted of the two types of AD (20 intrinsic and 3 extrinsic AD patients), and their positive frequencies of Ni, Co and Cr were 40, 35 and 20% in the intrinsic AD patients, and 33.3, 0 and 0% in the extrinsic AD patients. The positive frequencies of one or more metals were 65 and 33.3%, respectively.

Table 2
Frequencies of positive patch tests to metals using three different PTMs.

	Nickel	Cobalt	Chrome
PTM-1	10/35 (28.6%)	10/35 (28.6%)	8/35 (22.8%)
PTM-2	6/35 (17.7%)	4/35 (11.4%)	3/35 (8.6%)
PTM-3	6/16 (37.5%)	4/16 (25.0%)	3/16 (18.8%)
Total	22/86 (25.6%)	18/86 (20.9%)	14/86 (16.3%)

PTM: Patch test material.

Table 3

Frequencies of positive patch tests to metals in intrinsic and extrinsic AD patients.

	Nickel	Cobalt	Chrome	At least one metal
Intrinsic	13/31 (41.9%)	12/31 (38.7%)	7/31 (22.6%)	19/31 (61.3%)
Extrinsic	9/55 (16.4%)	6/55 (10.9%)	7/55 (12.7%)	14/55 (25.5%)
Fisher's exact test	$P=0.019$	$P=0.005$	$P=0.243$	$P=0.001$

When we divided 54 patients into two groups, the Ni, Co, Cr, or one or more positive group, and the negative group, there were no significant differences in SCORAD or VAS of pruritus. Thus, the severity of eruptions did not affect metal patch test positivity.

It was reported that FLG deficiency may represent a risk factor for contact sensitization to allergens, such as Ni [14]. Therefore, the frequencies of positive metal patch test were analyzed in relation to FLG mutation. In the total patients, there was no significant difference in the patch testing results of three metals between the FLG mutation-bearing and non-bearing groups (Table 4). Even when analysis was limited to extrinsic AD, no difference was found between FLG mutation-positive and negative groups.

3.3. High Ni concentration in sweat from intrinsic AD patients

In 17 AD patients (extrinsic, 12; intrinsic, 5), sweat was collected from the forearms by exercise, and the amount of Ni and Co in the sweat was measured. The concentration of Ni was significantly higher in the sweat of intrinsic AD than extrinsic AD patients (Fig. 2a). When the sweat Ni concentration was analyzed as the function of serum IgE values, there was an inverse correlation between them (Fig. 2b). In Fig. 2, patients with positive and negative Ni patch test results are shown as closed and open rectangles. Notably, one patient with negative Ni patch test in the intrinsic AD group had a low Ni concentration in the sweat.

4. Discussion

The intrinsic type of AD, characterized by unimpaired skin barrier, is enigmatic in its causes and pathophysiology. Immunologically, both extrinsic and intrinsic AD patients may exhibit increased production of IL-5 and IL-13 in the blood or skin, but when peripheral lymphocytes are stimulated with anti-CD3 antibody, extrinsic AD patients have a decreased capacity to produce IFN- γ [34]. Accordingly, we have shown that the circulating IFN- γ ⁺ Th1 cell frequency is higher in intrinsic than extrinsic AD. There are tendencies that Th2 and Th17 cell

frequencies are higher in extrinsic and intrinsic AD patients, respectively [34]. Thus, all the data collectively suggest that intrinsic AD has a relatively Th1-preponderant state as compared with extrinsic AD.

The normal barrier function and Th1-activating potency of intrinsic AD [15,35] raise the possibility that the patients are not sensitized with protein allergens capable of inducing Th2 responses, but with other antigens. It is assumed that protein antigens are not allowed to penetrate the skin of intrinsic AD with normal barrier. Instead, non-protein antigens, such as metals [19], can enter through the barrier and may induce Th1 responses. In 137 atopic children, 19.3% patients were positive to metals [20], although the two types of AD were not separately studied. In 1965, Shanon reported a patient with metal allergy exhibiting a skin manifestation indistinguishable from AD under the name of "pseudo-atopic dermatitis" [19,36].

In this study, we used three different PTMs, which gave similar but considerably variable positive patch test results. Nevertheless, we found that intrinsic AD showed positive patch tests to Ni and Co at higher percentages than extrinsic AD. Metals such as Ni, Co and Cr are known to cross-react with each other [37,38]. Cross reactivity may occur in our patch tests depending on the concentration of metals and the moieties of vehicles. The frequency of positive patch tests to at least one of the three metals was more than twice higher in intrinsic than extrinsic AD, suggesting that metal allergy is one of the potential causes of intrinsic AD. Interestingly, Co allergy is more prevalent in females than males [15], which is in accordance with the female preponderance of intrinsic AD.

Haptens and metals are representatives of non-protein, small antigenic molecules. The mechanisms underlying Th1-polarizing action of metals remain unclear. Recently, Ni has been shown to activate Toll-like receptor 4 (TLR4) signaling in antigen presenting cells (APCs) such as dendritic cells [39]. The same TLR4 stimulation occurs with Co, and the necessity of histidines H456 and H458 of human TLR4 is evident for activation of APCs by Co [40]. Thus, metals can interact with not only major histocompatibility/self peptide complex [41] but also with TLR4. TLR4 stimulation induces NF- κ B activation and conversion of proIL-1 β to IL-1 β [39,40], which has no skewing ability to Th1 or Th2 cells. Accordingly, Ni, Co, and Cr show a mixed Th1- and Th2-type cytokine response in peripheral T cells from sensitized patients [42], which is different from Th2-stimulatory protein antigens [11].

The high incidence of contact sensitization to metals in AD patients can be explained by three mechanisms [20]. First, barrier dysfunction of stratum corneum, as represented by FLG mutation, allows allergens to penetrate below the stratum corneum [43]. FLG is histidine-rich polypeptide, which chelates Ni in the epidermis [44]. However, we did not find a difference in the positive metal patch test between FLG mutation positive and negative patients, as reported in another study [45]. Rather, the positive incidence tended to be higher in FLG mutation negative patients. Therefore, metal allergy is unlikely associated with the barrier condition. Second, individuals highly ingesting or exposed to metals in daily life possibly develop AD via metal allergy. It has been shown that environmental Ni exposure is more important than genetic disposition, such as FLG mutation, in the development of Ni allergy [46]. Finally, APCs expressing TLR4 in AD skin might be different from normal APCs and prone to TLR4-mediated stimulation.

Metals are administered with foods and applied to the skin with jewelry [22,47]. Personal habits may increase the risk of development of contact dermatitis to metals. For example, women show a higher sensitization rate to Ni than men perhaps by wearing Ni-containing jewelry [48], which might result in the female preponderance of Ni allergy in intrinsic AD. It was reported

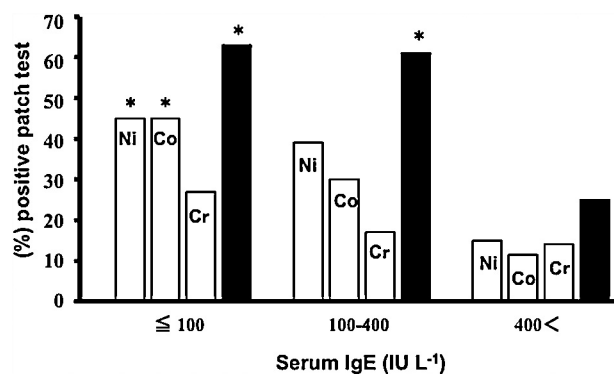


Fig. 1. Relationship between IgE level subgroups and frequencies of positive metal patch tests. The closed bars represent the positive patch test to one or more of the three metals. * $P < 0.05$, compared with the corresponding the 400 < groups.

Table 4Frequencies of positive patch tests to metals in patients with or without *FLG* mutation.

	Total AD patients				Extrinsic AD patients			
	Nickel	Cobalt	Chrome	At least one metal	Nickel	Cobalt	Chrome	At least one metal
<i>FLG</i> mutation positive patients	1/10 (10.0%)	1/10 (10.0%)	1/10 (10.0%)	2/10 (20.0%)	1/9 (11.1%)	0/9 (0.0%)	0/9 (0.0%)	1/9 (11.1%)
<i>FLG</i> mutation negative patients	9/39 (23.1%)	9/39 (23.1%)	6/39 (23.1%)	15/39 (38.5%)	2/20 (10.0%)	2/20 (10.0%)	2/20 (10.0%)	3/20 (15.0%)
Fisher's exact test	<i>P</i> =0.663	<i>P</i> =0.663	<i>P</i> =1	<i>P</i> =0.459	<i>P</i> =1	<i>P</i> =1	<i>P</i> =1	<i>P</i> =1

that only piercing women in AD group had a higher incidence of sensitization to Ni but AD patient without piercing had no increased risk of Ni allergy [33]. In our study, 40% of female intrinsic and 13% of female extrinsic AD patients had piercing histories. Piercing is not so popular in Japan as European and US countries, as piercing is usually prohibited in Japanese high schools. Given this circumstance, the piercing frequency may be high in intrinsic AD.

Ni or Co-rich food items include peanuts, hazelnuts, almond, chocolate, cocoa, sunflower seeds, beans, dried beans, porridge oats, licorice, lucerne seeds, oat meal, wheat bran [49–51]. Excess intake of these foods allows metal ions to be extraordinarily administered. In addition, aging, taking dietary supplement, and drinking tap water contribute to the total load of Ni in body [22,47]. Ni is present in most of daily foods and an average diet supplies 0.3–0.6 mg of Ni to the body [52]. Nickel concentration range between $2.348 \pm 1.716 \text{ mg kg}^{-1}$ in nuts and 0.002 mg L^{-1} in water [53]. Intestinal absorption of food is thought to be about 1% [54]. Accordingly, serum Ni levels correlate with Ni-rich food items. When Ni allergic patients avoided Ni intake, their serum Ni levels were

lowered compared to controls [48]. It is thought that metals are excreted through sweat, and therefore, sweating possibly may elicit dermatitis by serving as contactants. We found that the concentration of Ni was higher in the sweat of intrinsic than extrinsic AD patients, suggesting that metal allergy may be more significant in intrinsic AD.

In our study, the patients' sweat samples were collected from the forearms. Eczematous lesions preferentially occur on the flexor aspects of the limbs and around the neck of AD patients [55]. The degree of sweat secretion has been variously reported in AD [56]. It is also unclear that sweating is different between lesional and non-lesional skin of AD patients. There is an observation that the lesional skin has poor amount of sweat caused by dysfunction of autonomic nervous system [55]. It is an issue, therefore, that the metal concentration is uniform or different in the various skin sites [57]. The possibility remains that Ni concentration is different depending on the skin sites, which might explain the predilection areas of intrinsic AD.

Metal-free diet and elimination of metals improve skin eruptions in some AD patients [19,36]. Direct association between the metal concentrations of sweat and serum is an important issue to be clarified. It has been reported that serum Ni levels correlate with Ni-rich food items. When Ni allergic patients avoided Ni intake, their serum Ni levels were lowered compared to controls [48]. In addition, in 6 patients with palmar pompholyx showing positive patch tests to Ni, Co and Cr, oral metal challenge deteriorated their eruptions [58]. Further investigation on metal concentrations of food, serum and sweat might clarify the important aspect of pathophysiology of intrinsic AD, which is possibly evoked by Th1-inducing substances represented by metals.

Funding source

This work was supported by Grants-in Aid for Science Research from the Ministry of Health, Labour, and Welfare of Japan; and the Ministry of Education, Science, Sports, and Culture of Japan.

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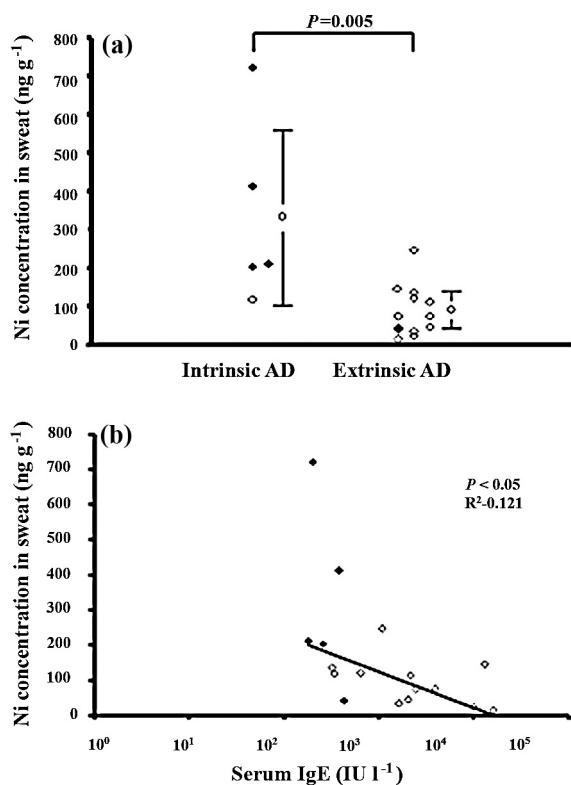


Fig. 2. Concentration of Ni in sweat of intrinsic and extrinsic AD patients. (a) Concentration of Ni in sweat from intrinsic and extrinsic AD patients. (b) Inverse correlation between Ni concentration and serum IgE levels. The closed and open rectangles represent patients with positive and negative Ni patch test.

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