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Original Article

Distinct *SPINK5* and *IL-31* polymorphisms are associated with atopic eczema and non-atopic hand dermatitis in Taiwanese nursing population

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Abstract: The term 'hand dermatitis' describes inflammatory skin condition localized to the hands. Nurses working at hospital settings are prone to develop hand dermatitis. The current study aimed to evaluate whether certain genetic polymorphisms were associated with the development of atopic eczema or non-atopic hand dermatitis in Taiwanese population. Nurses of Kaohsiung Medical University Hospital were recruited. Atopic eczema, non-atopic hand dermatitis and normal control groups were identified. The serine protease inhibitor Kazal type 5 (*SPINK5*), filaggrin and interleukin-31 (*IL-31*) gene variants were compared between the diseased and control groups. Our results showed that rs2303070 T allele of *SPINK5* (assuming recessive model; OR = 3.58, 95% CI 1.63–7.84; $P = 0.0014$) and rs7932 G allele of *IL-31* (assuming recessive model; OR = 18.25, 95% CI = 3.27–101.94; $P = 0.0009$) were associated with increased risk of developing atopic eczema, while rs6892205 G allele of *SPINK5* (assuming dominant model; OR = 3.79, 95% CI 1.55–9.28; $P = 0.0036$) was associated with the development of non-atopic hand dermatitis. In summary, our results showed that distinct *SPINK5* and *IL-31* gene variants were associated with the development of atopic eczema and non-atopic hand dermatitis. The barrier function, particularly those regulated by *SPINK5*, may play an important role in the development of both atopic eczema and non-atopic hand dermatitis.

Key words: atopic eczema – interleukin 31 – non-atopic hand dermatitis – serine protease inhibitor Kazal type 5

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The term 'chronic hand dermatitis' describes prolonged, non-infectious inflammatory skin condition localized to the hands. Many factors, including genetic and environmental components, have been proposed to contribute to the development of hand dermatitis (1). Atopic eczema, a disease characterized by barrier defect and immune dysfunction, is a well-established risk factor associated with the occurrence of hand dermatitis (2,3). It has been suggested that as much as one half of the patients with hand dermatitis can be considered atopic patients. In addition, the clinical patterns of hand dermatitis observed in atopic and non-atopic individuals had been reported to be very similar (4). Therefore, adult patients presenting with chronic hand dermatitis were often suspected to be patients with atopic constitution. More recently, it was recognized that atopic hand eczema may have a distinct morphology characterized by the involvement of skin lesions confined to the dorsal hand surfaces and volar wrists while sparing the palmar areas (5–7). In terms of genetic predisposition, it has been shown that after controlling for atopic eczema, the effect of genetic risk factors explained 41% of the variance in liability for developing hand dermatitis (8). This result indicated that a genetic component not associated with atopic eczema contributed significantly to the development of hand dermatitis.

It is currently believed that an impaired barrier function may be crucial for the development of hand dermatitis owing to the increased penetration of potential allergens and reduced resistance against irritants (9,10). Therefore, both defects in skin barrier integrity and exposure to environmental allergens/irritants contributed to the development of hand dermatitis. Of particular interest is the association found between certain variants of barrier function genes, including filaggrin (*FLG*) and serine protease inhibitor Kazal type 5 (*SPINK5*), as well as inflammatory mediator gene interleukin-31 (*IL-31*) with the occurrence of either atopic eczema or irritant hand dermatitis in different studies (11–13). These previously published association studies, however, did not analyse the potential differences between atopic eczema and non-atopic hand dermatitis.

Healthcare workers are prone to develop hand dermatitis that can result in lost work time, increased medical costs, and hampered life quality (14,15). Nurses, in particular, are prone to develop hand dermatitis because of the nature of their job that requires repetitive hand hygiene (3,16). Therefore, this special group was selected as our study population to evaluate whether certain genetic polymorphisms were associated with the development of atopic eczema or non-atopic hand dermatitis in Taiwanese population.

Materials and methods

Study population

A total of 1218 nursing staff from Kaohsiung Medical University Hospital were invited to participate in the study. The study was approved by the institutional review board of Kaohsiung Medical University Hospital (KMUH-IRB-960217), and informed consent was given by the participants. Of the invited nursing staff, 1132 completed the study. For the diagnosis of hand dermatitis, a validated questionnaire was employed. Briefly, a questionnaire comprising 13 questions was used to evaluate the signs and symptoms, the duration and severity, and the differential diagnoses of hand dermatitis. Diagnosis of hand dermatitis was made based on the established diagnostic algorithm (3,15,17). Atopic eczema was evaluated by physician diagnosis using Hanifin and Rajka criteria (18). Accordingly, the phenotypes of the participants were categorized into three groups: (i) the atopic eczema (n = 90; 43 with concomitant hand dermatitis and 47 without hand dermatitis), (ii) the non-atopic hand dermatitis (n = 205) and (iii) a control group with no atopic eczema and no hand dermatitis (n = 837). The studied population consisted of 99.4% (1125) women. For genetic analysis, 52 patients from the atopic eczema, 61 patients from the non-atopic hand dermatitis and 250 nurses from the control group agreed to participate.

Marker selection

We selected 10 single nucleotide polymorphisms (SNPs) of three genes from the HapMap database based on the common disease–common variant hypothesis (minor allele frequency of ≥0.10; Han Chinese in Beijing) and relevant findings from the literature. Four previously identified missense *SPINK5* SNPs with high occurring frequencies were identified. More specifically, rs6892205 Q267R (19), rs2303064 D386N 9 (13,19–21), rs2303067 K420E (13,20,21) and rs2303070 E825D (20,21) within SPINK5 were selected. For *FLG* SNPs, we included missense SNP rs1[illegible]24340 P478S, which was reported to be associated with the occurrence of psoriasis in Taiwanese population (22). We also selected three additional *FLG* SNPs based on the occurring frequencies as documented by HapMap Chinese population. More specifically, the association between two missense SNPs, rs2184953 Y2194H and rs11204987 S1482Y, and one synonymous SNP, rs3126066 D3497D, with the development of atopic eczema/non-atopic hand dermatitis was evaluated. Finally, two IL-31 SNPs, rs4758680 and rs7977932, were selected based on similar principle.

Genotyping

Venous whole blood was drawn from the participants, and DNA was extracted using standard published methods (Gentra Puregene® DNA Purification Kit; Gentra Systems, Minneapolis, MN, USA). SNPs were genotyped using TaqMan SNP Genotyping Assay with Applied Biosystems 7900HT Fast Real-Time PCR Systems (Foster City, CA, USA). This assay was designed for allelic identification of specific SNPs. For each genotyping experiment, 10 ng of DNA sample was added to TaqMan Genotyping Master Mix® to obtain a total volume of 10 μl solution. For the analysis, the SDS version 2.2 software (Life Technologies, Foster City, CA, USA) was employed. This software used an advanced multicomponent algorithm to calculate the distinct allele signal generated from the fluorescence measurement obtained during the assay plate reading. The sample genotypes were then automatically determined, and a cluster plot with visualized data across entire sample was generated. The frequencies of our data set in Taiwanese control subjects were near identical to the HapMap Han Chinese samples. This result indicated that the results obtained from our analyses were unlikely due to bias.

Statistical analysis

The statistical analyses were carried out using SAS version 9.20 (SAS Institute Inc, Cary, NC, USA). Clinical profiles between atopic and non-atopic hand dermatitis were compared using chi-square tests. Descriptive statistics were performed using generalized linear regression models for continuous variables. Genotypic frequencies were compared between case and control samples. The associated P-values were calculated using standard chi-square tests. Multiple testing-adjusted P-values were determined using the Bonferroni correction method (considering 10 SNPs; cut-off of P = 0.005). We tested for phenotype–genotype associations using dominant (i.e. both mutant-homozygotes and heterozygotes have the same disease ORs) and recessive (i.e. the heterozygotes have no higher disease ORs than the wild type) models as appropriate. We analyzed the OR of atopic eczema or non-atopic hand dermatitis for the carriers of at-risk genotypes using multiple logistic regression models. Lastly, we carried out statistical analyses to calculate linkage disequilibrium (LD) coefficients (r^2 and D′) using Haploview *v4.2* (23).

Results

Atopic nurses with hand dermatitis reported significantly increased occurrences of moderate to severe pruritus as compared to the non-atopic individuals

Of the 1132 participants, 248 nurses were identified to have hand dermatitis. More specifically, 43 have concomitant atopic eczema and 205 were non-atopics according to the physician diagnosis. Further analyses comparing the clinical profiles of hand dermatitis between the atopic and non-atopic individuals revealed that the atopic nurses with hand dermatitis were more likely to have moderate/severe itching sensations as compared to their non-atopic counterparts. No significant difference was found in terms of blister/fissure formation, burning sensation or frequencies of rash (Table 1).

Distinct genotype–phenotype associations

The demographic data of the participants for the genetic association study are shown in Table 2. Accordingly, significant differences were found in terms of age, work hours, work years and housework hours between the three groups. These parameters were considered as potential confounders and were adjusted for during subsequent analyses. No differences were found in terms of recruitment from different work sectors (i.e. special care unit), which was found to be a risk factor for the development of hand dermatitis in our previous study (15). We next compared the distributions of the allelic and genotypic frequencies of 10 SNPs among the selected genes including *SPINK5*, *FLG* and *IL31* (See Table S1). Our results demonstrated that the distribution of the selected SNPs was in accordance with the expected Hardy–Weinberg equilibrium among the control group. A significant association was observed between *SPINK5* rs6892205 Q267R [G] and the non-atopic hand dermatitis group (P = 0.00086). On the other hand, the *SPINK5* rs2303070 E825D [T] was associated with atopic eczema group (P = 0.0044) but not with non-atopic hand dermatitis group. Among the 61 non-atopic nurses with hand dermatitis, six mutant-homozygotes and 42 heterozygotes carrying

Table 1. Clinical symptoms and signs of hand dermatitis nurses with or without atopic eczema

Clinical symptoms/sign	Atopic patients with hand dermatitis (*n* = 43) Age: 31.7 ± 5.8 years *N* (%)	Non-atopic patients with hand dermatitis (*n* = 205) Age: 32.4 ± 7.1 years *N* (%)	*P*-value
Itching			
None or mild	20 (46.5)	136 (66.3)	**0.01**
Moderate or severe	23 (53.5)	69 (33.7)	
Blisters			
Never/almost never	21 (48.8)	120 (58.5)	0.24
Sometimes or frequently	22 (51.2)	85 (41.5)	
Rashes			
Never/almost never	8 (18.6)	61 (29.8)	0.14
Sometimes or frequently	35 (81.4)	144 (70.2)	
Burning sensation			
Never/almost never	25 (58.1)	138 (67.3)	0.25
Sometimes or frequently	18 (41.9)	67 (32.7)	
Cracks and/or fissures			
Never/almost never	11 (25.6)	44 (21.5)	0.56
Sometimes or frequently	32 (74.4)	161 (78.5)	

No significance was found in terms of age between the hand dermatitis groups with or without atopic eczema.
All atopic participants were women, while two of 205 non-atopic hand dermatitis participants were men.
Bold value indicate $P < 0.05$.

Table 2. Demographic information of participants for genetic association study

	Control (*n* = 250)	Non-atopic hand dermatitis (*n* = 61)	Atopic eczema (*n* = 52)	
	Mean (SD)	Mean (SD)	Mean (SD)	*P*-value
Age	28.58 (5.83)	30.92 (6.[illegible])	30.41 (7.19)	**0.0112**
Work year(s)	6.35 (5.24)	8.12 (6.53)	[illegible]5)	**0.0147**
Work day(s)	5.63 (0.57)	5.57 (0.53)	5.67 (0.51)	0.6301
Work hour(s)	46.44 (6.88)	44.38 (5.5[illegible])	47.[illegible]7.08)	**0.0363**
Housework hour(s)	2.68 (4.94)	4.87 (9.0[illegible])	4.33 (9.18)	**0.0306**

SD, standard deviation.
**P*-values from generalized linear regression models for continuous variables and from chi-square tests for categorical variables.
All participants are women.
Bold value indicate $P < 0.05$.

rs6892205 G allele were found. Because the presence of G allele at rs6892205 was associated with significant risk of developing non-atopic hand dermatitis, dominant effect of G allele was suggested. Subsequently, assuming a dominant model, we showed that G allele at rs6892205 dramatically increased the risk of developing non-atopic hand dermatitis (OR = 3.79, 95% CI 1.55–9.28; $P = 0.0036$) after adjusting for covariates (See Table S2). On the other hand, for rs2303070, only mutant-homozygotes with at-risk T allele showed significant association with the development of atopic eczema. Subsequently, assuming a recessive model, the multivariate-adjusted OR of atopic eczema was 3.58 (95% CI 1.63–7.84; $P = 0.0014$) compared with the reference genotype carriers (See Table S2). The *IL-31* rs7977932 was associated with atopic eczema phenotype ($P = 0.00019$). After adjusting for covariates and assuming a recessive model, the corrected *P*-value was 0.0009 (OR = 18.25, 95% CI = 3.27–101.94; See Table S2). As both

Table 3. Frequency of *SPINK5* variants in rs6892205 Q267R and rs2303070 E825D

	Codon 267R		Codon 825D		Not 267R or 825D	
	n	Frequency %	*n*	Frequency %	*n*	Frequency %
Non-atopic hand dermatitis	55	90.16	3	4.92	3	4.92
Atopic eczema	27	[illegible]	13	25.00	12	23.08

SPINK5, serine protease inhibitor Kazal type 5.
Patients had one Codon 267R: heterozygote + mutant-homozygote) or two risk allele (Codon 825D: mutant-homozygote).
Variants of codon 267 and 825 were mutually exclusive in non-atopic hand dermatitis and atopic eczema groups.

SPINK5 rs2303070 and *IL31* rs7977932 were associated with the development of atopic eczema, potential interactions between these two SNPs were examined. No significant interaction was found (P for interaction >0.05). In regard to *FLG* gene variants, we did not observe any significant association between the selected SNPs with atopic eczema/non-atopic hand dermatitis phenotype. To confirm that atopic eczema and non-atopic hand dermatitis were in fact distinct entities from the genetic perspective, we analysed the conditions in which a participant may carry at-risk variants at both rs6892205 (significant association with non-atopic hand dermatitis) and rs2303070 (significant association with atopic eczema) for *SPINK5* gene. As shown in Table 3, no individual carried both at-risk variants ($P < 0.0001$) in this study. This result strengthened and validated the notion that distinct *SPINK5* variants were associated with atopic eczema and non-atopic hand dermatitis.

Discussion

Through genetic association analyses, this study demonstrated that certain *SPINK5* and *IL31* gene variants were significantly associated with the occurrence of atopic eczema. In accordance with current understanding of atopic eczema, skin barrier dysfunction was associated with atopic skin and offered a reasonable explanation for the higher occurrence of hand dermatitis observed among atopic patients. *SPINK5* encodes a serine protease inhibitor that is responsible for Netherton syndrome, a rare genetic disorder that includes the features of atopic eczema (24). Dysfunction of *SPINK5* may result in abnormal keratinocyte differentiation and disturbed skin barrier function (25). Atopic eczema has been associated with *SPINK5* polymorphisms in studies carried out on different ethic populations (13,20,21), although some have not been able to replicate similar association (26,27). A British and two Japanese studies have previously shown that certain rs2303067 variant was associated with the development of atopic eczema (13,20,21). In our study, this SNP only showed a marginal significance ($P = 0.028$). It should be noted that previous studies have suggested potential parent-of-origin effect and association of this gene variant with serum IgE levels (13,21,28,29). However, our study design did not allow for further analysis addressing these possibilities. On the other hand, rs2303070 variant showed significant association with atopic eczema in this study. The functional significance of this variant remains to be elucidated in the future. Intriguingly, a distinct *SPINK5* variant was found to be associated

with the development of non-atopic hand dermatitis in this study. More specifically, our result indicated that rs6892205 G allele, which resulted in the substitution of the neutral Gln to basic Arg, was associated with the development of non-atopic hand dermatitis. Therefore, it is reasonable to hypothesize that this rs6892205 variant may have functional significance because of the remarkable changes in side chain acidity. Our previous study had identified the frequency of hand wash, a decontaminating procedure that can result in barrier disruption, as a behavioural risk factor associated with the development of non-atopic hand dermatitis among nurses, which is consistent with the current finding (3). Taken together, distinct *SPINK5* variants are associated with the development of atopic eczema and non-atopic dermatitis.

Interleukin-31 is a novel effector cytokine that is associated with atopic eczema-induced skin inflammation and itch in human beings (30,31). In transgenic mice overexpressing IL-31, the mice developed severe itching dermatitis but have normal IgE serum levels (32). In addition, anti-IL-31 antibody was found to ameliorate the scratching behaviour in mouse dermatitis model (33). Therefore, IL-31 is strongly associated with inflammatory skin condition characterized by intense itch. Association between *IL-31* gene variants and eczema has previously been demonstrated in three independent European populations (12). More specifically, in the study by Schulz et al. (12), they identified three main haplotypes for *IL-31* that accounted for 93.4% in their study population. Further analyses revealed that certain haplotype containing rs7977932 G allele is associated with the development of eczema particularly those of non-atopic nature as defined by the serum levels of specific IgE against common food and inhalant allergens. In this study, we found significant association between rs7977932 G allele under recessive model and the occurrence of atopic eczema, which was identified by careful physician diagnosis in our study. The different methods used for identifying atopic status likely contributed to the differences found between the results of previous study and ours'. It should be noted that the confidence interval of *IL-31* analysis is rather large. However, a closer examination of our results revealed that for atopic eczema group, the rs7977932 GG homozygote accounts for approximately 10% of the patients. On the other hand, rs7977932 GG homozygote was found in <1% of the control group. Therefore, *IL-31* rs7977932 is significantly associated with the development of atopic eczema in our study population. It is worthy to mention that while the clinical signs of hand dermatitis between atopic and non-atopic individuals were similar, atopic patients with atopic hand dermatitis reported more severe pruritus than their non-atopic counterparts. Because IL-31 is strongly associated with pruritus, it is likely that the *IL-31* polymorphism harboured by atopic individuals contributed to the enhanced severity of this symptom.

An unexpected result obtained from this study is that we did not find significant association between *FLG* polymorphisms and the development of atopic eczema. Certain gene variant encoding for *FLG*, the key protein for the development of the cornified envelope and the process of cornification, has been shown to predispose individuals for developing atopic eczema and hand dermatitis in various populations (6,34–36). More specifically, Palmer et al. (35) had previously shown that two independent loss-of-function genetic variants, namely the *R510X* and *2282del4*, in the *FLG* gene are strongly associated with atopic eczema in the European population. For Taiwanese population, the impact of these specific *FLG* mutations on the development of atopic eczema remained unclear. Previous studies focusing on *FLG* mutations in Taiwan have suggested that *R510X* and *2282del4* mutations are probably absent in Taiwanese population (22,37). To explore possible associations between other *FLG* polymorphisms and atopic eczema in Taiwanese population, the common variants of *FLG* were searched within the HapMap Chinese database based on common disease–common variant hypothesis. None of the selected variants, however, showed association with either atopic eczema or non-atopic hand dermatitis phenotype. As it has been shown that populations with different ancestral groups harboured their own unique set of *FLG* mutations (34), additional study employing direct sequencing approach is currently in progress to elucidate the potential associations between *FLG* mutations and atopic eczema/non-atopic hand dermatitis among the Taiwanese population.

The major strength of this study is that the status of atopic eczema was carefully evaluated by dermatologist. This clear phenotype distinction constituted the cornerstone that allowed for genotype distinction between two apparently similar yet intrinsically different skin conditions. Without such clear distinction, the intricate differences between atopic eczema and non-atopic hand dermatitis among our study population in terms of genetic predisposition will probably not be revealed. This study also has important limitations. First, the atopic eczema and the non-atopic hand dermatitis group harboured distinct *SPINK5* variants that were significantly different from the controls. How these *SPINK5* variants contribute to the skin barrier dysfunction requires additional studies in the future. Secondly, although our results suggested that skin barrier function, particularly those regulated by *SPINK5*, may play an important role in both atopic eczema and non-atopic hand dermatitis among nurses, the same phenomenon may not be true in different occupational settings owing to the differences in environmental exposure. Additional study addressing this complex issue is also warranted. Thirdly, the majority of the participants in this study were female nurses. How gender may affect our results needs to be further addressed. It should also be mentioned that only 363 of 1132 nurses agreed to participate in the genetic study which required taking blood samples. It is not surprising that studies involving invasive procedures (i.e. venipuncture) reduce the willingness of voluntary participation. A higher participation rate would certainly enhance the significance of our results and reduce the impact of potential confounders including selection bias. Last but not least, hand dermatitis may result from contact allergies or irritant exposures. The method employed in this study did not allow for distinction between these two factors. However, it has been noted that most cases of hand dermatitis among the nursing staff are irritant contact dermatitis (38–40), and therefore, our results are likely to be applicable to hand dermatitis resulting from irritant exposure.

In summary, we have demonstrated that distinct *SPINK5* and *IL-31* gene variants were associated with the development of atopic eczema and non-atopic hand dermatitis. The barrier function, particularly those regulated by *SPINK5*, may play an important role in both atopic eczema and non-atopic hand dermatitis. Because the development of both atopic eczema and non-atopic hand dermatitis involves intimate gene–environment

interaction, future studies should focus on how different genetic background interacts with relevant environment. Only through this approach may better treatment strategies evolve for these two frequently encountered and often debilitating skin conditions.

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Author contributions

Cheng-Che E. Lan and Gwo-Shing Chen performed and designed the research and wrote the paper; Hung-Pin Tu and Ching-Shuang Wu, Yi-Wei Lu, Wan-Chen Li, Yin Chun Chen performed the research and analysed the data; Ying-Chin Ko and [illegible] Yu designed the research.

Conflict of interest

The authors declare that there are no conflicts of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Genotypes distribution of *SPINK5*, *IL31* and *FLG* in non-atopic hand dermatitis and atopic eczema.

Table S2. Missense variants in *SPINK5* associated with non-atopic hand dermatitis and atopic eczema.

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