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ORIGINAL PAPER

Interactions between *FLG* mutations and allergens in atopic dermatitis

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Abstract Filaggrin gene (*FLG*) mutations and sensitization in patients with atopic dermatitis (AD) have been well documented. However, whether an interaction exists between these mutations and specific sensitization in AD patients is still unknown. The aim of the study was to explore the interaction between *FLG* mutations and specific sensitization in AD patients. A total of 249 AD outpatients were recruited in the current study. Skin prick tests were conducted to assess the patient's sensitization to specific allergens. *FLG* mutations were analyzed through comprehensive sequencing. Logistic regression analyses were conducted to determine the interactions between *FLG* mutations and sensitization present. The mean age of the patients was 3.5 years, and the mean age of onset of AD was 9.6 months. The mean SCORAD of the patients was 25.8. Fourteen types of mutations were identified in the *FLG* of 64 patients. A total of 24 (9.6 %) and 29 (11.6 %) cases were mutated with 3321delA and K4671X, respectively. Sensitization to at least one type of allergen was detected in 118 patients (47.4 %). Logistic regression analyses showed that *FLG* mutations presented an interaction with sensitization to peanut and did not interact with the other detected allergens among AD patients. Sensitization to peanut allergens would have an interaction with the mutation of K4671X and the combined mutations in *FLG* in patients with atopic dermatitis. However, sensitization to the other common allergens might not interact with *FLG* mutations in the development of atopic dermatitis.

Keywords Atopic dermatitis · Filaggrin gene · Mutations · Sensitization · Allergen

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Introduction

Atopic dermatitis (AD) is a common inflammatory skin disorder with complex etiology and pathogenesis [1]. Research shows that genetics plays a major role in determining the development of AD [6, 10, 15, 23, 29, 31, 37]. However, the increased prevalence of AD in recent years have suggested a complex interaction between environmental factors and susceptibility genes underlying the attack of this atopic disease [1]. Although this hypothesis is well known, no study has made substantial advances to prove it.

Recently, Bisgaard reported that filaggrin gene (*FLG*) mutations had a gene–environment interaction with cat exposure at birth in the development of early-life eczema,

but this interaction was absent in dog, mite, or *Staphylococcus aureus* exposure [3]. The interactions between *FLG* mutations and sensitization were not analyzed because allergic sensitization is uncommon in early life (less than 1-year old) and very few children are positive to skin prick tests (SPTs) [3]. More recently, Schuttelaar found an interaction between cat exposure and *FLG* mutations in AD [33]. This study also reported an association between the combined genotype of *FLG* mutations and specific IgE to birch allergen, wherein allergen sensitization was defined as the presence of IgE $\geq$ 0.70 kU/L against at least one tested specific allergen (such as house dust mite, mold, and so on) [33].

Nevertheless, these studies initially explored the presence of gene–environment interactions in AD patients. However, patients with AD are clinically known to have positive reactions to several allergens from the environment, such as food, inhalations, and microorganisms [8, 13, 27, 36]. The skin prick test is believed to have higher sensitivity and specificity in identifying sensitization to allergens compared with serologic methods, because the level of IgE varies largely among individuals [18, 28]. Moreover, the statistical power would drop when a continuous variable (the level of IgE in sera) is dealt with as a binary variable. We have recently analyzed the *FLG* mutations in Chinese AD patients and found a spectrum of *FLG* mutations that were different from those in Europeans [20, 37, 38]. In the present study, we examined the *FLG* mutations in Chinese AD patients and detected allergen sensitization using SPT to determine whether an interaction existed between *FLG* mutations and sensitization to common allergens in AD patients.

Methods

Subjects

In the present study, AD was diagnosed based on the criteria of Hanifin and Rajka [14]. The subjects were outpatients from the dermatological clinics of the Department of Dermatology, Shanxi Provincial Children's Hospital, and the Shanghai Jiaotong University School of Medicine. The combined occurrence of asthma and allergic rhinitis in AD patients was defined based on the questionnaire answers and previous medical records. All individuals in the present study were of Chinese Han ancestry. Written informed consents were obtained from all the participants and the parent/guardian involved in this study. The current study was approved by the Ethics Committee of the Shanghai Jiaotong University School of Medicine. This study was conducted in accordance with the principles of the Declaration of Helsinki.

FLG genotyping

Genomic DNA samples were extracted from peripheral whole blood using TIANamp Blood DNA kits (TIANGEN Biotech, Beijing, China). A comprehensive sequencing of the *FLG* gene in 249 AD patients was performed using an overlapping polymerase chain reaction (PCR) strategy, which enables the sequencing of the entire *FLG* coding sequence. The PCR primers and conditions have been described previously by Sandilands et al. [32]. The comprehensive sequencing of PCR products was conducted using an Applied Biosystems 3730 DNA analyzer (ABI incorporation, Carlsbad, California, USA).

Skin prick tests

The reactions in 249 AD patients to 15 common allergens were determined through SPT (Merck-KGaA, Germany) (Table 3). The skin (back or flexor side of the forearms) of the subjects was pricked. Histamine hydrochloride (10 mg/mL) and saline were used as the positive and negative controls, respectively. The results of the SPTs were read 15–20 min after the tests were performed. SPT positive reaction was characterized by a wheal with a geometric mean diameter of at least 3 mm greater than the negative control. SPTs were performed after the discontinuation of antihistamines and topical steroids for at least 7 days.

Statistical analysis

This is a case-only study [12]. The patients with *FLG* mutations were classified as case-like group and those without mutations as control-like group. A sensitization (positive reaction to allergens) tested by SPTs was defined as the exposure. The unconditional logistic model was used to estimate the multiplication model of interaction between the mutations and exposure after adjusting by associated variables (such as age and gender). The quantitative values in the study were expressed as mean ($\pm$SE) or median ($\pm Q$) and compared using the Student's *t* test or the Mann–Whitney *U* test in accordance with the data distribution. Frequencies and percentages were used to describe the categorical variable data. Significant differences in the genotype frequency among the analyzed groups were assessed using the Chi-square test or Fisher's exact test, as appropriate. Interactions between *FLG* gene mutations and the sensitization were determined through logistic regression analyses. All the estimates were reported with odds ratios (OR) and 95 % confidence intervals (95 % CI); $\alpha < 0.05$ was considered to indicate statistical significance. The statistical data were analyzed using the SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA). The statistical power analyses were conducted by the software

of Power Analysis and Sample Size (PASS, NCSS LLC, Kaysville, UT, USA) version 11.

Results

From January 2009 to December 2010, 249 unrelated AD patients were enrolled in the current study. Among these patients, 160 (64.3 %) individuals were male. The mean age of the patients was 3.5 (ranging from 0.5 to 30.5) years. The mean onset age of AD was 9.6 ± 1.4 (ranging from 0.25 to 180) months (Table 1). The SCORAD of the patients ranged from 5 to 76, with a mean of 25.8 ± 0.9.

FLG genotyping

After genotyping, 14 types of mutations were identified in the *FLG*. Five patients carried compound heterozygous mutations, three with 3321delA and K4671X, one with 4026delT and K4671X, and one with Q1790X and 7145del4. A total of 64 patients carried *FLG* mutation(s). The most common mutations in this set of patients were 3321delA (24 cases, 9.6 %) and K4671X (29 cases, 11.6 %) (Table 2).

Sensitization in AD

Of the 249 patients, SPT revealed that approximately 62 cases (24.9 %) were positive to hen's egg (white), followed by yolk with 45 cases (18.1 %), mugwort with 26 cases (10.4 %), cow's milk with 20 cases (8 %), weedmix with 17 cases (6.8 %), *Dermatophagoides pteronyssinus* with 15 cases (6 %), *D. farinae* with 14 cases (5.6 %) and peanut with 12 cases (4.8 %). Based on the SPT results, 118 patients (47.4 %) were sensitized to at least one kind of allergen (Table 3). In these 249 patients with AD, 2 cases were positively reactive to seven kinds of allergens, 3 cases reactive to six allergens, 5 cases reactive to five allergens, 7 cases reactive to four allergens, 19 cases reactive to three allergens, 38 cases reactive to two allergens and 44 cases reactive to one allergen.

Table 1 Demographic profiles of patients with atopic dermatitis

Profiles of patients with atopic dermatitis	
Male (*n*, %)	160 (64.3 %)
Age (mean ± SE, year)	3.5 ± 0.2
Age of onset (mean ± SE, month)	9.6 ± 1.4
SCORAD (mean ± SE)	25.8 ± 0.9
≤25 (*n*, %)	149 (59.8 %)
25–50 (*n*, %)	79 (31.7 %)
>50 (*n*, %)	21 (8.4 %)
Positive for atopic pedigree history (*n*, %)	81 (32.5 %)
Asthma (*n*, %)	26 (10.4 %)
Rhinitis (*n*, %)	105 (42.2 %)
Family history of AD (*n*, %)	73 (29.3 %)
Family history of asthma (*n*, %)	28 (11.2 %)
Family history of rhinitis (*n*, %)	142 (57.0 %)

Table 2 *FLG* mutations detected through comprehensive sequencing in patients with atopic dermatitis ($n = 249$)

Mutations in *FLG*	Cases (%)
3222del4	4 (1.6)
3321delA	21 (8.4)
3321delA and K4671X	3 (1.2)
4026delT and K4671X	1 (0.4)
4271delA	1 (0.4)
478insA	1 (0.4)
5757del4	1 (0.4)
6834del5	1 (0.4)
8001del4	1 (0.4)
E2422X	1 (0.4)
K4671X	25 (10.0)
Q1790X and 7145del4	1 (0.4)
Q2397X	2 (0.8)
Q2417X	1 (0.4)
Total	64 (25.7)

Table 3 Atopic dermatitis patients with positive reaction to allergens detected via skin prick tests

Allergens	Cases (*n*)	%
Mugwort	26	10.4
Dog epithelia	6	2.4
Hen's egg (white)	62	24.9
Yolk (yellow)	45	18.1
Lamb's quarters	13	5.2
Weedmix	17	6.8
Peanut	12	4.8
Dermatophagoides farinae (mite I)	14	5.6
Dermatophagoides pteronyssinus (mite II)	15	6.0
Candida albicans	10	4.0
Cat epithelia	7	2.8
Shrimp	6	2.4
Cow's milk	20	8.0
Alternaria tenuis	5	2.0
Walnut	4	1.6
Total	118	47.4

The interactions between *FLG* mutations and sensitization

The two common mutations of 3321delA and K4671X and the combined mutations in *FLG* were analyzed in the

interaction analyses. After adjustment by sex and age, logistic regression analyses showed that peanut sensitization had an interaction with the mutation of K4671X (OR = 4.045, $P = 0.039$) and combined mutation in *FLG* (OR = 3.496, $P = 0.042$). Peanut sensitization did not interact with the mutation of 3312delA (Table 4). Noticeably, neither the two most prevalent mutations nor the combined mutations in *FLG* showed any interaction with the other allergens detected by SPT (Table 4). We also conducted a logistic regression analysis between *FLG* mutations and the number of positive allergens identified in a single case and the result was negative (OR = 1.064, $P = 0.519$, adjusted by age).

Discussion

The pathogenesis of allergic/immune reactions is complex. Hence, the causation (allergen) for an individual with an allergic disease is difficult to reveal precisely. Food challenge is considered as the most informative method to detect food allergens; however, this method is usually difficult to conduct for both doctors and patients [26]. The level of IgE was thought to be index that reflects the allergy and could be easily detected via ELISA, but with low sensitivity. It is well known that type 1 and type 4 (late-phase reactions) hypersensitivity reactions participate in the development of AD [1, 9, 30]. SPT, as a major method to detect type 1 hypersensitivity, could detect the status of sensitization to both food and inhalation with a relatively higher sensitivity than the level of IgE [7]. In the present study, we analyzed the sensitization in AD patients and initially explored the interaction between *FLG* mutations and allergen sensitizations through SPT.

Among the patients with mild to moderate AD, 25.5 % carried one kind of *FLG* mutation. The frequency of *FLG* mutations in the present study was similar to that in a Japanese population (27 %) [25], higher than that in a Singaporean population (20 %) [6], but much lower than that in a well-studied European population (45 %) [5]. These differences may be accounted to subject selection. The patients in the present study had mild to moderate AD (SCORAD <50 in more than 90 % of the patients, Table 1), whereas the patients in previous studies had a moderate to severe AD [5, 6]. Another possible explanation of the difference would be racial difference between Chinese and Europeans, as reported by Chen et al. [6].

Studies on the interaction between *FLG* mutations and allergen sensitizations in the development of AD are still lacking. Bisgaard and Schuttelaar reported that an interaction existed between *FLG* mutations and neonatal cat exposure in the onset of eczema in infancy [3, 33]. However, we believe that the term "cat exposure" is not the same as sensitization to allergens from cat. Individuals exposed to cat may have more opportunities to attain contact with mites, cat epithelia and microorganisms. Thus, an interaction between cat exposure and *FLG* mutations might indicate an interaction between *FLG* mutations and

Table 4 Interactions between *FLG* mutations and allergens in atopic dermatitis analyzed through logistic regression adjusted by age and sex

	3321delA ($n = 24$)			K4671X ($n = 29$)			Combined mutations in *FLG* ($n = 64$)		
Allergen	OR	95 % CI	*P*	OR	95 % CI	*P*	OR	95 % CI	*P*
Mugwort	0.297	0.036–2.439	0.259	1.331	0.404–4.390	0.639	0.828	0.298–2.296	0.716
Dog epithelia	0	NA	NA	1.264	0.140–11.445	0.835	0.478	0.053–4.277	0.509
Hen's egg (white)	0.567	0.185–1.736	0.320	1.704	0.673–4.312	0.261	1.205	0.596–2.433	0.604
Yolk (yellow)	0.965	0.309–3.012	0.951	1.448	0.512–4.094	0.485	1.183	0.544–2.572	0.671
Lamb's quarters	0.713	0.081–6.285	0.761	1.236	0.248–6.166	0.796	1.327	0.365–4.823	0.667
Weedmix	0.446	0.053–3.718	0.455	1.466	0.383–5.606	0.576	1.059	0.344–3.259	0.920
Peanut	0.823	0.093–7.265	0.861	4.045	1.075–15.211	0.039	3.496	1.047–11.677	0.042
Mite I	1.689	0.333–8.559	0.527	2.105	0.545–8.130	0.280	1.899	0.577–6.253	0.291
Mite II	0.558	0.067–4.624	0.589	1.120	0.237–5.298	0.886	0.666	0.179–2.482	0.545
Candida albicans	0	NA	NA	1.862	0.364–9.514	0.455	0.786	0.159–3.893	0.768
Cat epithelia	0	NA	NA	1.179	0.134–10.333	0.882	0.485	0.056–4.194	0.511
Shrimp	5.182	0.807–33.262	0.083	0	NA	NA	1.421	0.250–8.083	0.692
Cow's milk	1.049	0.214–5.134	0.953	1.502	0.401–5.625	0.546	0.957	0.326–2.810	0.937
Alternaria tenuis	0	NA	NA	1.924	0.206–17.961	0.566	2.189	0.351–13.666	0.402
Walnut	0	NA	NA	0	NA	NA	0.868	0.087–8.648	0.904
Total	0.888	0.370–2.133	0.790	2.097	0.930–4.275	0.074	1.622	0.903–2.914	0.106

Mite I, *Dermatophagoides farinae*; Mite II, *Dermatophagoides pteronyssinus*; *NA* not analysis

allergens relative to cat. However, this hypothesis was not proven in the current study.

The results from logistic regression analyses indicated that only peanut allergen sensitization interacted with *FLG* mutations. The current study did not reveal any association between *FLG* mutations and cat epithelia. On the other hand, Bisgaard and Schuttelaar reported that an interaction existed between cat exposure in early life and *FLG* mutations in eczema or atopic disorder [3, 33]. In addition, we did not identify any other association between *FLG* mutations and the common allergen sensitizations in patients with AD, such as mites, egg, milk, shrimp and so on. These negative results may be attributed to the increase in water loss and dry skin caused by *FLG* mutations, which would make allergen invasion easier without selection (except for peanut). The current study did not reveal this association with this patient cohort (OR = 1.541, $P = 0.143$, Table 4). However, some animal experiments support the hypothesis that allergic sensitization in the atopic state occurs via transcutaneous or transmucosal passage of allergens facilitated by filaggrin deficiency [11, 17]. In addition, genetic causations other than *FLG* mutations may also exist. These genetic causations should be studied further.

In the present study, peanut was the only allergen sensitization found to interact with *FLG* mutations. No epidemiological data are available on peanut allergy in the Chinese population. In two other Asian populations, the prevalence of peanut allergy was 0.43–0.64 % [34], whereas it was 0.6–1.3 % in US adults [22, 35], and approximately 1.2–1.6 % in preschool and school age children [2, 35]. Although we only found 12 cases (4.8 %) with positive reaction to peanut, this percentage is significantly higher than that in the general population (1.4 %, using the median value between 1.2 and 1.6 %, $U = 4.585$, $P < 0.01$). The prevalence rates of peanut allergy in AD patients with or without *FLG* mutations were significantly higher compared with those in the general population: six cases (9.4 %) in patients with *FLG* mutations ($U = 5.430$, $P < 0.01$) and six cases (3.2 %) in patients without *FLG* mutations ($U = 2.105$, $P < 0.05$). Previous studies showed that the development of AD was associated with *FLG* mutations [29] as well as the peanut allergy [4, 19]. The findings of our study and the association between *FLG* mutations and peanut allergy reported by Brown et al. [4] indicated that the interaction between these two factors might contribute to the development of AD. Although we identified an interaction between sensitization to peanut and *FLG* mutations which would underlie the development of AD, the presence of either *FLG* mutations or peanut sensitization did not have any effect on the severity (SCORAD) of the patients (data not shown). Nemoto-Hasebe reported that *FLG* mutations were associated with SCORAD [24]. However, in our previous studies, we were unable to identify any association between SCORAD and mutations in the gene of *FLG* [21, 37], which was consistent with Hubiche's report [16].

More recently, Brown et al. [4] reported that the loss-of-function variants in *FLG* were a significant risk factor for peanut sensitization. Brown reported this association in a population of 71 individuals with peanut allergy from England, the Netherlands and Ireland, and then repeated this finding in a cohort of 390 individuals from Canada. Peanut allergy has been defined by Brown as positive food challenge, clinical history and SPT wheal to peanut >8 mm or peanut-specific IgE >15 kU/L. Brown recruited subjects from a longitudinal birth cohort and a peanut allergy cohort and analyzed the association controlling for coexistent AD. In the current study, we recruited AD patients as subjects and revealed that individuals with *FLG* mutations had significantly higher frequency of positive reaction to peanut allergens than those without *FLG* mutations. The values of the ORs in the logistic analyses indicated that AD patients with *FLG* mutations would increase the odds (approximate four times) of sensitizing to peanut compared to those without the mutations. Interestingly, this increased odds was only attributed to the mutation of K4671X. In the cohort of Brown's study, patients with peanut allergy, 62–85 % coexistent with AD, were positive, with *FLG* mutations in 16.9 % individuals [4]. In the present study, 50 % (6 in 12) of patients with peanut allergy had *FLG* mutations. Since the association between *FLG* mutations and peanut allergy is not solely attributable to the coexistence of AD [4], there is a probability that significant association between *FLG* mutations and peanut allergy is essential for the interaction. Further studies are needed to clarify this issue and the authors, however, remind the readers that caution is needed to understand the interaction we identified because of the not high statistical power.

There was no existing data that we could base on to predict the sample size when analyzing the interaction between *FLG* mutations and sensitization in AD. According to Table 4, 4 cases (13.8 %) out of the 29 individuals with mutation of K4671X showed a positive reaction to peanut sensitization in SPT, while this proportion was 3.7 % (8 cases) in the 216 individuals without mutation of K4671X. Assume that the significance level was 0.05 and a ratio of cases versus controls was 1:7, the statistical power for the interaction between K4671X and peanut sensitization was 62 % in the present study. We did not analyze the statistical power of the interactions of the other allergen sensitizations with *FLG* mutations because of the negative results. We could not exclude the presence of a possibility that the negative results were due to the small sample size in the present study. Nevertheless, the current study initially reported an interaction between *FLG* mutations and

peanut sensitization in AD patients. However, this interaction was not present when the most common allergens were analyzed. These findings are important in understanding the function impairment from *FLG* mutations and its action in the development of AD.

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