

ORIGINAL ARTICLE

Mutations analysis in filaggrin gene in northern China patients with atopic dermatitis

M. Li,[†] Q. Liu,[‡] J. Liu,[§] R. Cheng,[†] H. Zhang,[†] H. Xue,[‡] Y. Bao,[¶] Z. Yao^{†,*}

[†]Department of Dermatology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

[‡]Department of Dermatology, Shanxi Provincial Children's Hospital, Taiyuan, Shanxi, China

[§]Department of Dermatology, Bao'an Maternal and Child Health Hospital, Shenzhen, Guangdong, China

[¶]Department of Pediatrics, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

*Correspondence: Z. Yao. E-mail: zyaosmu@sohu.com

Abstract

Background Recently, we have reported filaggrin mutations (*FLG*) of atopic dermatitis in southern China. However, there have been few detailed reports of *FLG* mutations of patients with AD in northern China by now.

Objectives The present aim was to establish the mutation spectrum of *FLG* gene in AD patients in northern China.

Methods A total of 339 cases met Hanifin and Rajka diagnostic criteria of AD were recruited. A comprehensive sequencing of the entire *FLG* coding region in these patients was conducted. All detected *FLG* null mutations were screened in a cohort of 301 normal controls.

Results Seven novel mutations (478insA, Q1070X, 4026delT, Q1712X, Q2397X, 7145del4 and 8001del4) and eleven reported mutations (3222del4, 3321delA, 4271delAA, S1515X, Q1790X, 5757del4, 6834del5, Q2417X, E2422X, 7945delA and K4671X) in AD were identified. Mutations 3321delA and K4671X were two of the most common mutations in AD. *FLG* null mutations were present in 26.0% of AD patients. *FLG* null alleles (compound genotypes) were significantly higher in AD ($P < 0.001$) than in the controls. The compound genotypes for all *FLG* variants were significantly associated with IV ($P < 0.001$) and palmar hyperlinearity ($P < 0.001$). The common mutation, K4671X, was significantly associated with AD-coexistent allergic rhinitis ($P = 0.005$).

Conclusions Our study increases the total number of *FLG* mutations. We clearly demonstrated that *FLG* loss-of-function mutations were significantly associated with AD in northern China. The *FLG* null mutations in the Chinese population differed not only from that in the European population but also from that in sub-populations of Asians outside of the Chinese mainland.

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Conflict of interest

The authors have declared that they have no conflict of interest.

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Introduction

Atopic dermatitis (AD) is a common inflammatory skin disorder characterized by epidermal barrier dysfunction.¹ Filaggrin gene (*FLG*) null variants as strong predisposing factors for AD have been demonstrated and replicated in multiple independent case-control studies in different populations.^{2–7} However, there were two negative results of *FLG* null mutation associated with AD. In 2008, Giardina *et al.* genotyped two common *FLG* mutations (R501X and 2282del4) in European populations, and found that these two null mutations did not confer susceptibility to AD in Italian patients.⁸ To determine whether other mutations located

elsewhere in *FLG* confer risk to AD, they performed a full sequencing of *FLG* in 220 Italian patients. They only found three novel *FLG* null mutations and a number of variations, but they were not associated with AD.⁹

Ma *et al.* genotyped eight *FLG* mutations (E2422X, Q2417X, S2554X, S2889X, S3296X, R4307X, 3321delA and 7945delA) in AD patients in northern China and only found that a strong association of 3321delA mutation with AD. The frequency of this mutation was 15.0%.¹⁰ Our previous study in Shanghai (southern China) showed that *FLG* null mutations are strongly associated with AD patients.⁴ The frequency of *FLG* null mutation was

31.4%, and mutations 3321delA and K4671X were the most common mutations in Chinese AD patients. The frequency of mutation K4671X was 9.2%. This mutation, however, could not be found in Taiwanese and Singaporean Chinese AD and ichthyosis vulgaris (IV) patients.^{11,12} Most of these patients had moderate-to-severe AD (91.4%) in our previous study.⁴ To confirm our previous findings and further elucidate the *FLG* mutations in northern Chinese patients with AD, we selected another AD cohort of 399 patients in Shanxi province to sequence the *FLG* gene. 75.2% of the patients had mild-to-moderate AD, and the other 24.8% of patients have severe AD. Taken together, there might be further prevalent *FLG* mutations to be discovered in the northern China patients with AD.

Materials and methods

This study was approved by the Ethics Committees of the Shanghai Jiaotong University School of Medicine and the Children's Hospital of Shanxi, China. Written informed consents were given by all the participants including AD patients and healthy controls before enrollment in this study. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Study population

A total of 339 unrelated northern China patients with AD who met the AD criteria of Hanifin and Rajka were recruited.¹³ These patients were outpatients referred to dermatological clinics at the Department of Dermatology, the Children's Hospital of Shanxi. The combined occurrence of asthma and allergic rhinitis in the 339 AD patients was defined on the basis of the questionnaire answers and a previous diagnosis by physicians or otolaryngologists.

To compare the frequency of *FLG* mutations, DNA samples from 301 normal healthy, unrelated Chinese individuals were also included.

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 the 339 AD patients was performed using an overlapping PCR strategy, which enables the sequencing of the entire *FLG* coding sequence. PCR primers and conditions were described previously by Sandilands *et al.*¹⁴ The comprehensive sequencing of PCR products was conducted on an Applied Biosystems 3730 DNA analyser (ABI incorporation, Carlsbad, CA, USA). A total of 301 healthy controls were screened for all the *FLG* mutations detected in 339 AD patients.

Skin prick tests

Skin prick tests (SPTs) were performed on 251 AD patients to determine their reactions to 18 common allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, egg albumen, egg

yolk, peanut, mugwort, cat dander, mixed grass and tree pollen, among others, all from Merck-KGaA, Germany). The skin (back or flexor side of the forearms) of the patients 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 to 20 min after the tests were performed. Skin prick test positive reaction was defined as a wheal with geometric mean diameter of at least 3 mm. SPTs were performed after the discontinuation of antihistamines and topical steroids for at least 7d.

Statistical analysis

Descriptive statistics for quantitative values were expressed as mean ($\pm$ SD) 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. The statistical significance of differences in genotype frequency among analysed groups was assessed using a chi-square test or Fisher's exact test, as appropriate. Logistic regression analyses were performed to detect the association between mutations in the *FLG* gene and the AD-associated phenotypes, including asthma, allergic rhinitis, etc. The strength of association was estimated by calculating the odds ratio (OR) with a 95% confidence interval (CI). As a quality check for the genotyping, genotype data were analysed for deviations from the Hardy-Weinberg equilibrium using chi-squared statistics. All alleles were observed to be in normal Hardy-Weinberg equilibrium. The level of statistical significance was established at $\alpha < 0.05$. All statistical data were analysed using the SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA). The following effect was detected at a power of 0.75 and at $\alpha = 0.05$: OR = 2.3 compared with the normal controls.

Results

Clinical features

A total of 339 patients presented AD symptoms that corresponded with the Hanifin and Rajka diagnostic criteria. The clinical features of the patients are outlined in Table 1. The median ages between boys and girls were not different. However, the boys' age of onset was younger than that of the girls (2.0 ± 3.5 months vs. 2.5 ± 11.0 months, $P = 0.019$). In addition, the percentage of AD combined with asthma in boys was significantly higher than that in girls ($\chi^2 = 5.367$, $P = 0.021$). No significant difference was found between boys and girls for the other combined phenotypes in AD (Table 1). The average age of the 301 controls (104 girls and 197 boys) was 13.90 ± 4.37 years.

FLG mutations in AD patients

Seven novel mutations, namely, 478insA, Q1070X, 4026delT, Q1712X, Q2397X, 7145del4, and 8001del4 and 11 reported mutations, namely, 3222del4, 3321delA, 4271delAA, S1515X, Q1790X, 5757del4, 6834del5, Q2417X, E2422X, 7945delA and K4671X,

Table 1 Descriptive characterization of patients with AD in northern China

Variable	AD <i>n</i> = 339			Statistic	<i>P</i>
	Total	Male <i>n</i> = 221	Female <i>n</i> = 118		
Age (M ± QR, years)	2.0 ± 4.0	1.5 ± 4.0	2.5 ± 4.0	<i>Z</i> = 1.845	0.065
Age of onset (M ± QR, months)	2.0 ± 4.0	2.0 ± 3.5	2.5 ± 11.0	<i>Z</i> = 2.336	0.019
SCORAD (M ± QR)	24.0 ± 19.9	23.5 ± 21.1	24.2 ± 15.4	<i>Z</i> = 0.277	0.782
Mutation in <i>FLG</i> (heterozygous)	76 (22.4)	44 (19.9)	32 (27.1)	χ^2 = 2.712	0.100
Mutation in <i>FLG</i> (homozygous)	12 (3.5)	6 (2.7)	6 (5.1)	χ^2 = 0.986	0.321
Combined with					
Asthma	35 (10.3)	29 (13.1)	6 (5.1)	χ^2 = 5.367	0.021
Rhinitis	134 (39.5)	93 (42.1)	41 (34.7)	χ^2 = 1.732	0.188
Ichthyosis vulgaris	110 (32.5)	69 (31.2)	41 (34.7)	χ^2 = 0.436	0.509
Xerosis	258 (76.1)	164 (74.2)	94 (79.7)	χ^2 = 1.258	0.262
Palmar hyperlinearity	18 (5.3)	10 (4.5)	8 (6.8)	χ^2 = 0.778	0.378
Orbital darkening	54 (15.9)	34 (15.4)	20 (16.9)	χ^2 = 0.141	0.708
Keratosis pilaris	67 (19.8)	39 (17.6)	28 (23.7)	χ^2 = 1.794	0.18
White dermographism (<i>n</i> = 334)	141 (41.6)	92 (41.6)	49 (41.5)	χ^2 = 4.85×10^{-5}	0.994
Cheilitis	21 (6.2)	16 (7.2)	5 (4.2)	χ^2 = 1.193	0.275
Scalp dermatitis	158 (46.6)	109 (49.3)	49 (41.5)	χ^2 = 1.879	0.17
Infra-auricular and retroauricular fissuring	127 (37.5)	78 (35.3)	49 (41.5)	χ^2 = 1.275	0.259
Family history of AD	104 (30.7)	63 (28.5)	41 (34.7)	χ^2 = 1.408	0.235
Family history of asthma	38 (11.2)	25 (11.3)	13 (11.0)	χ^2 = 0.007	0.935
Family history of rhinitis	187 (55.2)	121 (54.8)	66 (55.9)	χ^2 = 0.043	0.835
Positive skin prick test (<i>n</i> = 251)	120 (47.8)	79 (35.7)	41 (34.7)	χ^2 = 0.285	0.593

Values within parenthesis are expressed in percentages.

AD, atopic dermatitis; *FLG*, Filaggrin gene; QR, interquartile range; SCORAD, scoring atopic dermatitis.

were identified. Mutations K4671X and 3321delA were the most common mutations in this AD cohort. The data on *FLG* mutations are summarized in Table 2.

FLG mutations were predisposing factors for AD in northern China patients

About 26% of Chinese AD patients carried one or more *FLG* mutations in this cohort (combined minor allele frequency 14.7, *n* = 678). These variants were also carried by 3.99% of the Chinese control individuals (combined minor allele frequency 0.02, *n* = 602). The associations between AD and the two most common mutations (3321delA and K4671X) were both statistically significant (3321delA: OR = 9.8, 95% CI: 3.0–31.5, *P* = 1.68×10^{-6} , K4671X: OR = 4.2, 95% CI: 2.0–8.9, *P* = 2.91×10^{-5}). The rarer *FLG* variants were non-significantly associated with AD when analysed individually. However, the combined null genotype showed a highly significant association (OR = 6.5, 95% CI: 3.6–11.7, *P* = 2.16×10^{-14}).

The association between the *FLG* mutations and AD-associated phenotypes, including asthma, allergic rhinitis, xerosis, palmar hyperlinearity, orbital darkening, keratosis pilaris, white dermographism, cheilitis, scalp dermatitis, infra-auricular retroauricular fissuring, family history of atopy and positive skin prick test, was also assessed. The compound genotypes for all *FLG* variants were significantly associated with IV (OR = 2.5, 95% CI: 1.8–3.6,

P = 2.67×10^{-7}) and palmar hyperlinearity (OR = 2.5, 95% CI: 1.7–3.9, *P* = 4.72×10^{-4}). No significant association was observed between the compound genotypes and the other AD-associated phenotypes (Fig. 1). However, the common mutation, K4671X, was significantly associated with AD-coexistent allergic rhinitis (χ^2 = 7.849, *P* = 0.005).

In a linear regression analysis, the compound genotypes for all *FLG* variants were non-significantly associated with the total SCORAD (24.2 ± 15.9 vs. 23.5 ± 20.4, *P* = 0.343). Moreover, no difference was found between the median age of onset of individuals carrying *FLG* mutations and those not carrying any *FLG* mutations (2.0 ± 3.0 months vs. 2.0 ± 5.0 months, *P* = 0.307).

Discussion

Atopic dermatitis had increased in prevalence in recent decades, both in developed and developing countries. These diseases have been recognized as complex traits resulting from an interaction between gene defects and environmental stimuli.^{15,16} During the past few years, a strong association between *FLG* defects and allergic disorders has been observed in different populations.^{2–7} Filaggrin is a key protein that plays an important role in the formation of the protective skin barrier, and protects the organism against environmental agents.¹⁷ Several observations indicate that the absorption of allergens through the skin in patients with *FLG*

Table 2 Atopic dermatitis case-control association analysis for *FLG* null variants in northern China patients with AD

FLG mutation	FLG genotype		OR (95% CI)	χ^2	P-values
	Control (n = 301)	AD (n = 339)			
478insA	0	1 (0.3%)	–	–	–
3222del4	1 (0.33%)	5 (1.5%)	4.4 (0.5–37.8)	1.180	0.277*
3321delA	3 (1.00%)	33 (9.7%)	9.8 (3.0–31.5)	22.931	1.68×10^{-6}
Q1070X	0	1 (0.3%)	–	–	–
4026delT	0	1 (0.3%)	–	–	–
4271delAA	0	1 (0.3%)	–	–	–
S1515X	0	1 (0.3%)	–	–	–
Q1712X	0	1 (0.3%)	–	–	–
Q1790X	0	1 (0.3%)	–	–	–
5757del4	0	3 (0.9%)	–	1.116	0.291*
6834del5	0	1 (0.3%)	–	–	–
7145del4	0	1 (0.3%)	–	–	–
Q2397X	0	2 (0.6%)	–	–	0.501†
Q2417X	0	3 (0.9%)	–	1.116	0.291†
E2422X	0	1 (0.3%)	–	–	–
7945delA	0	2 (0.6%)	–	–	0.501†
8001del4	0	1 (0.3%)	–	–	–
K4671X	8 (2.66%)	38 (11.2%)	4.2 (2.0–8.9)	17.478	2.91×10^{-5}
Combined‡	12 (3.99%)	88 (26.0%)	6.5 (3.6–11.7)	58.384	2.16×10^{-14}

Short lines indicated insufficient data for reliable statistical analyses.

*Continuity correction.

†Fisher's exact test.

‡Combined of the mutations above.

AD, atopic dermatitis; FLG, Filaggrin gene.

defects may be a predisposing factor for the development of other allergic disorders.¹⁸

To date, approximately 60 loss-of-function *FLG* mutations have been identified in European and Asian populations.^{3–7,11,12,19,20} In the current study, seven novel and eleven reported mutations were identified. These variants led to non-sense or frameshift mutations, which either prevented or severely diminished the production of free *FLG* in the epidermis.⁷ The associations between AD and the two most common mutations (3321delA and K4671X) were both statistically significant. However, the rarer *FLG* variants were non-significantly associated with AD when analysed individually. It may be because the sample size of AD patients in this study was small. Mutations 3321delA and K4671X were the two most common mutations in the AD patients in northern China. Mutation 3321delA was also the most common mutation in other Asian populations. Mutation K4671X, however, was not the common mutation in these populations.^{11,12} In a recent study, Chen *et al.* screened 92 Singaporean Chinese individuals with IV and/or moderate-to-severe AD, and K4671X was not found in this cohort.¹² In our previous study, we found that the frequency of K4671X mutation was 9.2% in southern China. Perhaps K4671X is a common mutation specific to the Chinese mainland population. The mutation 3222del4 was also common mutation in northern

China. We found five AD patients and one healthy control harbouring this mutation. The seven novel mutations were not found in 301 normal controls and our AD patients in southern China, and it indicated that these mutations may be unique in AD patients in northern China. The reported rare mutations (4271delAA, S1515X, Q1790X, 5757del4, 6834del5, Q2417X, E2422X and 7945delA) were also found in AD patients in other Asian populations. These mutations may be family specific. The most common mutations in European populations, namely, R501X and 2282del4, were not found in the cohorts of the current study. These results indicate that the *FLG* null mutation in the Chinese population differed not only from that in the European population but also from that in sub-populations of Asians outside of the Chinese mainland.

Among the 339 AD patients, more male (221) than female (118) patients had ongoing AD. The boys' age of onset was younger than that of the girls. A slightly higher prevalence among male patients has been reported in our previous study and that of Böhme *et al.*^{21,22} Halkjaer *et al.* reported that the age of peaked prevalence rate for girls was older than that for boys.²³ These results indicate that males may be more susceptible to the atopic disorders. However, the reasons for such increased susceptibility remain unclear. A census or a random sample of a child population may be needed to clarify this issue.

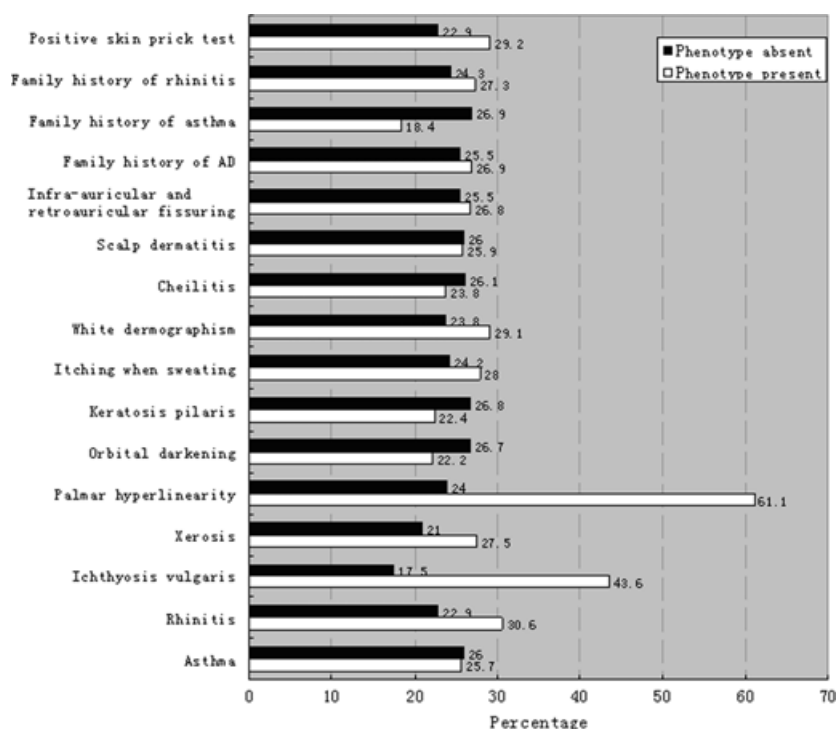


Figure 1 Comparison of the frequencies of *FLG* gene mutations in patients with AD with phenotypes vs. those without the phenotypes.

In the AD cohort, the compound genotypes for all *FLG* variants were found significantly associated with IV and palmar hyperlinearity. This result is easily understandable because the *FLG* gene is the pathogenic gene of IV. *FLG* mutations were present in 25.7% of 35 AD patients associated with atopic asthma and in 26.0% of total AD patients. No significant association was found between them, indicating that the phenotype of asthma in AD has no effect on the association between *FLG* null mutations and AD. This result is consistent with that of our previous study.⁴ The *FLG* compound genotypes were not associated with allergic rhinitis. However, the common mutation, K4671X, was significantly associated with AD-coexistent allergic rhinitis ($\chi^2 = 7.849$, $P = 0.005$). No significant association was found between the compound genotypes and allergic sensitization, as shown in the analysis of the skin prick test results. This finding is consistent with those of a Polish cohort.²⁴

In conclusion, *FLG* null variants were found to have strong association with AD patients in northern China. Furthermore, the *FLG* mutations of Chinese AD patients were different from not only those of European and Japanese populations but also from those of Taiwanese and Singaporean Chinese populations.

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