

Mutations in the filaggrin gene in Han Chinese patients with atopic dermatitis

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To cite this article: Zhang H, Guo Y, Wang W, Shi M, Chen X, Yao Z. Mutations in the filaggrin gene in Han Chinese patients with atopic dermatitis. *Allergy* 2010; DOI: 10.1111/j.1398-9995.2010.02493.x.

Keywords

asthma; atopic dermatitis; filaggrin gene; mutation; skin barrier.

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Accepted for publication 7 September 2010

DOI:10.1111/j.1398-9995.2010.02493.x

Edited by: Stephan Weidinger

Abstract

Background: Recently, loss-of-function mutations in the filaggrin gene (*FLG*) have been identified as the cause of ichthyosis vulgaris (IV) and also predisposing factors for atopic dermatitis (AD) and AD-associated phenotypes. Until now, over thirty mutations in *FLG* have been identified in patients with IV and AD, but the *FLG* mutation spectrum is not clear for Chinese Han patients with AD. This study aimed to investigate the role of *FLG* mutations in AD and AD-associated phenotypes in a Chinese population.

Methods: We carried out a comprehensive sequencing of the entire *FLG* coding region in 261 Han AD patients.

Results: In our research, we identified ten novel mutations (R826X, 3222del4, R1140X, 4271delAA, Q1790X, 5757del4, 6834del5, 6950del8, S2706X and K4671X) and eight reported mutations (441delA, R501X, 3321delA, R1474X, Q2417X, E2422X, 7945delA and R4306X) in *FLG*. *FLG* mutations were present in 31.4% of our AD patients. Mutations 3321delA and K4671X, with a frequency of 14.6% and 9.2%, respectively, were two of the most common mutations in this AD cohort. *FLG* null alleles (compound genotypes) were significantly associated with AD ($P < 0.001$) and AD associated with food sensitization ($P = 0.012$). However, we did not observe a positive association between *FLG* mutations and other AD-associated phenotypes, including asthma, allergic rhinitis and elevated total serum IgE level.

Conclusion: Our study increases the total number of reported *FLG* mutations. Moreover, we further confirm that *FLG* mutations are strong predisposing factors for AD in Han Chinese.

Atopic dermatitis (AD), also known as eczema, is a chronic relapsing skin disease and frequently associated with other atopic conditions, such as asthma and allergic rhinitis. Over the past decades, the prevalence of AD increased steadily in developed countries, with 15–30% of children and 2–10% of adults affected (1). Over 3% of preschool children in China suffered from AD. Atopic dermatitis has long been recognized as a complex trait in that interactions between genes and environmental stimuli and interplay between multiple genes contribute to disease manifestation. Until now, the exact pathogenesis of AD remains unknown. More recently, a disturbance of epidermal barrier function that culminates in dry skin seems to be one of the central aspects in the pathophysiology of AD (2, 3). In 2006, the key role of protein filaggrin within the granular cell layer in maintaining the skin

barrier was determined followed by the identification of loss-of-function mutations in the gene encoding filaggrin (*FLG*) (4). These loss-of-function genetic variants have also been shown a major predisposing factor for AD (5).

During the past few years, a strong association between the *FLG* mutations and AD was observed in different populations (6–10). Filaggrin null mutations were also reported to confer significant risks of allergic sensitization and allergic rhinitis as well as asthma in the context of eczema (11, 12) and increased asthma severity in children and young adults (13). A cohort study of adult patients with persistent AD demonstrated that identification of *FLG* null alleles is an indicator of a poor prognosis in AD (14). Filaggrin mutations were also significantly associated with AD in Chinese (15).

The human profilaggrin gene is located on chromosome 1q21 and consists of three exons (16, 17). Exon 1 (15 bp) and 2 (159 bp) are small, whereas exon 3 is unusually large (12573 bp) and encodes most of the N-terminal S100 domain, B domains and 10–12 filaggrin repeats. The homology between the filaggrin repeats at the DNA level is almost 100%, making the conventional PCR and sequence analysis of the entire regions of exon 3 difficult. Because an overlapping polymerase chain reaction strategy was proposed (18), many other study groups could complete the comprehensive sequencing of the entire *FLG* coding regions. Until now, there have been identified over 30 *FLG* mutations in the previous studies, but none was first identified in Chinese patients with AD. In this study, using an overlapping PCR strategy, we have investigated the role of the mutations in *FLG* in patients with AD in a Han Chinese population and found novel mutations in *FLG*, some of which may be specific to Chinese. Furthermore, up to now, in all the studies that conducted comprehensive sequencing of the entire *FLG* coding region, this study has the largest sample.

Methods

Study population

Two hundred and sixty-one unrelated Chinese patients with AD in total who met the AD criteria of Hanifin and Rajka (19) were involved in this study. All the patients with AD were referred to outpatient dermatological clinics at the Department of Dermatology, Xinhua Hospital affiliated to Shanghai Jiaotong University School of Medicine, China. At the same time, we recruited 92 unrelated healthy Han Chinese individuals without history of AD, any atopic manifestations and IV as controls. Written informed consent was given by all the patients. All individuals in this study were of Han ancestry. This study was approved by the medical ethical committees of Shanghai Jiaotong University school of medicine, China.

Procedures

The clinical diagnosis of AD was made by two experienced dermatologists, who also performed a thorough clinical examination and recorded a complete medical history using a standardized questionnaire. Asthma and allergic rhinitis were defined on the basis of the questionnaire answers and a previous diagnosis by physicians or otolaryngologists. The severity of disease was evaluated using the widely accepted SCORing Atopic Dermatitis (SCORAD) index (20), which categorizes patients into mild (0–24 points), moderate (25–50 points) and severe (51–103 points). Venous blood samples were collected from part of patients with AD and analyzed for total serum IgE level, serum eosinophilic cationic protein (ECP) level and specific IgE antibodies including inhalant mix (hx2 contains *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, house dust and cockroach) and food mix (fx5E contains milk, egg, soybean, peanut, morrhua and wheat) by Pharmacia UniCAP-100 automatic immunoassay analyzer (Pharmacia Diagnostics AB, Uppsala, Sweden). An elevated total

serum IgE level was taken to be greater than 100 IU/ml, and an elevated serum ECP level was greater than 8 µg/l.

FLG genotyping

Genomic DNA samples were extracted from peripheral whole blood using TIANamp Blood DNA kits (TIANGEN Biotech, Beijing, China). We carried out a comprehensive sequencing of *FLG* in 261 patients with AD using an overlapping PCR strategy, which allows a sequencing of the entire *FLG* coding sequence. PCR primers and conditions were as described previously by Sandilands et al. (18). The comprehensive sequencing of PCR products was conducted on an Applied Biosystems 3730 DNA analyzer (ABI incorporation, Carlsbad, California, USA). We screened 92 healthy controls for all the *FLG* mutations detected in patients with AD in this study.

Statistical analysis

All statistics were analyzed with SPSS 17.0 software package (SPSS Inc., Chicago, Illinois, USA). Descriptive statistics for quantitative values were given as mean ($\pm$ SD). We used the Fisher's exact test to assess the associations between the *FLG* mutations and AD as well as AD-associated phenotypes, including asthma, allergic rhinitis, elevated total serum IgE levels, elevated ECP levels, food sensitization and inhalant sensitization. A linear regression analysis was performed to evaluate the association between *FLG* mutations and the SCORAD. Independent-sample *t*-test was used to compare the mean values of the age of onset of AD. The standard alpha level of 0.05 was considered statistically significant.

Results

Clinical features

Two hundred and sixty-one patients with AD all met the AD criteria of Hanifin and Rajka, whose clinical features were outlined in Table 1. The average age of 92 cases of healthy controls, with 37.0% women and 63.0% men, was 16.49 ± 1.75 years.

FLG mutations in patients with AD

We carried out a comprehensive sequencing of the entire *FLG* coding sequence in 261 patients with AD in this study. In total, we identified ten novel mutations, R826X, 3222del4, R1140X, 4271delAA, Q1790X, 5757del4, 6834del5, 6950del8, S2706X and K4671X (Figs 1 and 2) and eight reported mutations, 441delA, R501X, 3321delA, R1474X, Q2417X, E2422X, 7945delA and R4306X (Figs 1 and 2) in our AD cohort.

Among the ten novel mutations, five were categorized as frameshift mutation, introducing a downstream premature termination codon and five were categorized as nonsense mutation (Table 2). We also identified eight recurrent *FLG* mutations reported in the previous studies, of which three were categorized as frameshift mutation and five were

Table 1 Descriptive characterization of patients with atopic dermatitis (AD) in this study

Variable	Patients with AD
Han Chinese, <i>n</i> * (%)	261/261 (100)
Male sex, <i>n</i> * (%)	147/261 (56.3)
Early age of onset (<2 year), <i>n</i> * (%)	234/261 (89.7)
AD with asthma, <i>n</i> * (%)	121/261 (46.4)
AD with allergic rhinitis, <i>n</i> * (%)	112/261 (42.9)
Elevated total serum IgE (>100 IU/ml), <i>n</i> * (%)	162/224 (72.3)
Elevated serum ECP (>8 µg/l), <i>n</i> * (%)	170/176 (96.6)
fx5E positive, <i>n</i> * (%)	120/188 (63.8)
hx2 positive, <i>n</i> * (%)	125/186 (67.2)
Mild AD (SCORAD, 0–24 points), <i>n</i> * (%)	25/261 (19.6)
Moderate AD (SCORAD, 25–50 points), <i>n</i> * (%)	182/261 (69.7)
Severe AD (SCORAD, 51–103 points), <i>n</i> * (%)	54/261 (20.7)
Age (year), mean ± SD (range)	7.11 ± 8.39 (0.50–57.75)
Age of onset (year), mean ± SD (range)	1.46 ± 4.40 (0.02–36.00)
Total serum IgE (IU/ml), mean ± SD (range)	1242.66 ± 2901.51 (4.41–24600.00)
Serum ECP (µg/l), mean ± SD (range)	83.04 ± 59.82 (5.08–200.00)

*Number affected/total number with data available. SCORAD, SCORing atopic dermatitis; ECP, eosinophilic cationic protein.

categorized as nonsense mutation (Table 2). Mutations K4671X and 3321delA were two of the most common mutations in our AD cohort. The data of *FLG* mutations in our AD cohort were summarized in Table 2.

We also performed screening of eighteen *FLG* mutations detected in this study in 92 unrelated healthy controls without history of AD, any atopic manifestations and IV. None of the eighteen mutations were found in the controls.

FLG mutations were predisposing factors for AD in China

A total of 82 (31.4%) cases of patients with AD were identified with at least one *FLG* mutation, and none of the eighteen mutations were found in 92 healthy controls. A strong association between the combined *FLG* null variant genotypes and AD was observed (OR 1.458, 95% confidence intervals 1.343–1.583, $P < 0.001$) (Table 3). The combined minor allele frequency in the patients with AD was observed to be 0.195. Mutations 3321delA and K4671X were two of the most common mutations in our AD cohort, and the associations between these two mutations and AD were both statistically significant (3321delA: OR 1.413, 95% CI 1.316–1.516, $P < 0.001$; K4671X: OR 1.388, 95% CI 1.298–1.485, $P = 0.004$, Table 3).

We also assessed the association between the *FLG* mutations and AD-associated phenotypes, including asthma, allergic rhinitis, elevated serum total IgE, ECP levels and allergic sensitization. The compound genotypes for all *FLG* variants were significantly associated with food sensitization in the context of AD ($P = 0.012$, Table 3). However, we did not observe a significant association between the compound

genotypes and other AD-associated phenotypes, such as concomitant asthma, allergic rhinitis and elevated total serum IgE levels ($P > 0.05$, Table 3). Mutation 3321delA happened much more frequently in AD patients without asthma than those with asthma, and a significant association between mutation 3321delA and AD with asthma was observed (20% vs 8.3%, OR 0.360, 95% CI 0.167–0.777, $P = 0.016$, Table 3).

In a linear regression analysis, *FLG* mutations (compound genotypes) as well as mutations 3321delA and K4671X were not significantly associated with the total SCORAD and the objective SCORAD ($P > 0.05$, data not shown).

Following an independent-sample *t*-test, we found that the average age of onset of those individuals carrying heterozygous and homozygous or compound heterozygous *FLG* mutations was much younger than that of those not carrying any *FLG* mutations (0.52 ± 1.57 year vs 1.88 ± 5.15 year, $P = 0.001$, 95% CI -2.191 to -0.527). This indicated that individuals carrying *FLG* mutations had a much earlier age of onset of AD. Similarly, the patients with AD carrying mutation K4671X also had a much earlier age of onset of AD when comparing with those not carrying mutation K4671X (0.35 ± 0.48 year vs 1.57 ± 4.60 year, $P < 0.001$, 95% CI 0.598 – 1.835).

Discussion

In 2001, genetic linkage of AD to chromosome 1q21 was identified (21). This chromosome region named epidermal differentiation complex (EDC) contains a number of genes that fulfill important functions in terminal differentiation of the human epidermis (22). Filaggrin is also known as filament-aggregating protein, and its gene is one of the most important genes in EDC involved in terminal differentiation. The initial product of *FLG*, profilaggrin, is the main constituent of the keratohyalin granules in the granular cell layer of the epidermis. It is a 400-kilodalton polyprotein and consists of 10–12 tandem repeats of the filaggrin peptides. Profilaggrin is initially expressed inactively and subsequently proteolytically processed into individual functional filaggrin peptides of approximately 37-kilodalton. During the terminal differentiation of granular cells, filaggrin peptides rapidly aggregate the keratin cytoskeleton, causing collapse of the granular cells into flattened anuclear squames. Subsequently, within the stratum corneum (SC), the filaggrin peptides are progressively degraded by post-translational modification enzymes into a pool of hydrophilic amino acids, which together with their metabolites and various ions make up the natural moisturizing factor (NMF). Immunological and biochemical evidence showed that both profilaggrin and processed filaggrin were completely absent in patient carrying *FLG* mutations (4). *FLG* deficiency or absence results in reduced NMF and impaired epidermal barrier function, which are both likely contributors in the etiopathogenesis of AD. A very recent study revealed that the *FLG* mutations were associated with an increased skin pH (23).

During the past few years, *FLG* mutations have been widely identified in European, American, Austrian, Japanese

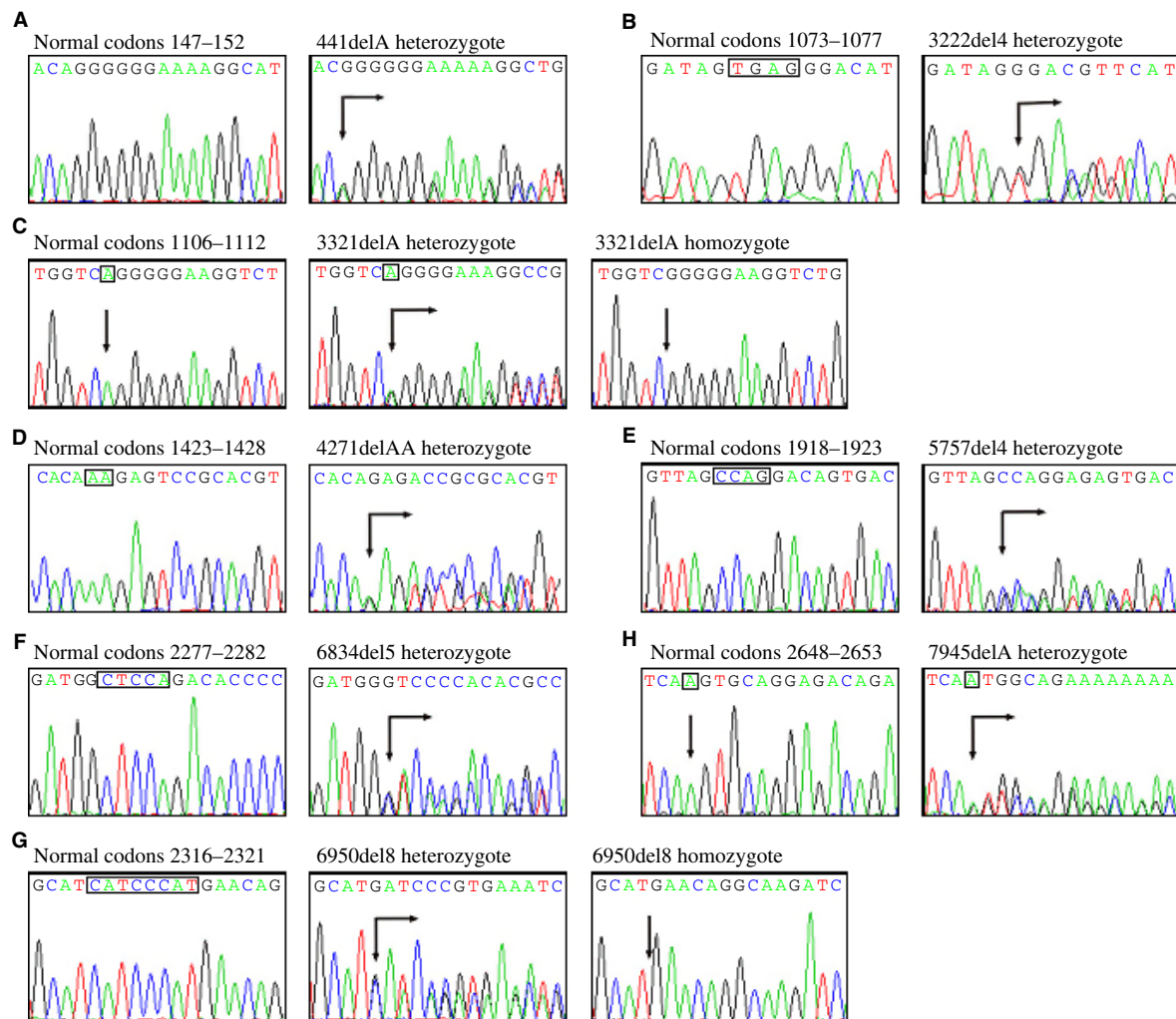


Figure 1 Frameshift mutations in filaggrin (*FLG*) detected in patients with atopic dermatitis (AD). Direct DNA sequencing of specific *FLG* PCR products. (A) Left panel shows the normal sequence from the *FLG* S100 domain corresponding to codons 147-152, and right panel shows the detection of a heterozygous 441delA mutation. (B) Left panel shows the normal sequence from *FLG* repeat 2 corresponding to codons 1073-1077, and right panel shows the detection of a heterozygous 3222del4 mutation. (C) Left panel shows the normal sequence from *FLG* repeat 2 corresponding to codons 1106-1112. Middle panel shows the detection of a heterozygous 3321delA mutation, and right panel shows a homozygous 3321delA mutation. (D) Left panel shows the normal sequence from *FLG* repeat 3 corresponding to codons 1423-1428, and right

panel shows the detection of a heterozygous 4271delAA mutation. (E) Left panel shows the normal sequence from *FLG* repeat 5 corresponding to codons 1918-1923, and right panel shows the detection of a heterozygous 5757del4 mutation. (F) Left panel shows the normal sequence from *FLG* repeat 6 corresponding to codons 2277-2282, and right panel shows the detection of a heterozygous 6834del5 mutation. (G) Left panel shows the normal sequence from *FLG* repeat 6 corresponding to codons 2316-2321. Middle panel shows the detection of a heterozygous 6950del8 mutation, and right panel shows a homozygous 6950del8 mutation. (H) Left panel shows the normal sequence from *FLG* repeat 7 corresponding to codons 2648-2653, and right panel shows the detection of a heterozygous 7945delA mutation.

and Taiwanese. For the first time, we reported a *FLG* mutation spectrum in Han Chinese patients with AD in this study. In total, we identified ten novel mutations and eight reported mutations in *FLG* and brought the total number of *FLG* mutations reported to date over forty. *FLG* mutations previously reported seemed to be population specific. Several *FLG* mutations, i.e. mutations R501X and 2282del4, were very common in the European population (18) but

rarely found in the Japanese (24) or in the Taiwanese population (25). Mutation R501X was detected in 4 of 174 southern Chinese patients with AD (26) and found in one patient with AD in our study, indicating it is European specific and rare in Asian population. Mutations 441delA, Q2417X, 7945delA and R4306X were also found in our patients with AD, which were once suggested to be unique to Singaporean Chinese (27). It was because that

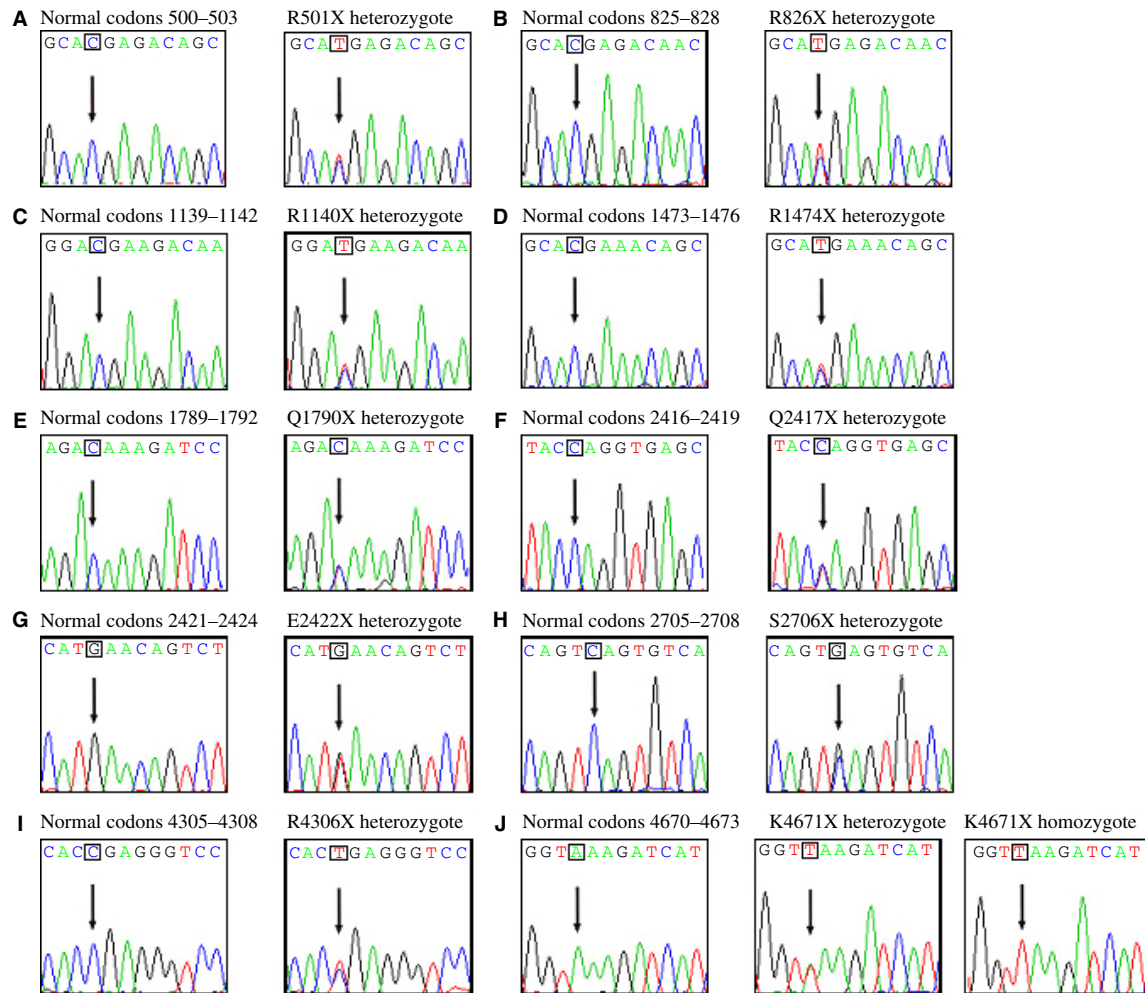


Figure 2 Nonsense mutations in filaggrin (*FLG*) detected in patients with atopic dermatitis (AD). Direct DNA sequencing of specific *FLG* PCR products. (A) Left panel shows the normal sequence from *FLG* repeat 1 corresponding to codons 500–503. Right panel shows a heterozygous transition mutation 1501C → T resulting in nonsense mutation R501X. (B) Left panel shows the normal sequence from *FLG* repeat 2 corresponding to codons 825–828. Right panel shows a heterozygous transition mutation 2476C → T resulting in nonsense mutation R826X. (C) Left panel shows the normal sequence from *FLG* repeat 3 corresponding to codons 1139–1142. Right panel shows a heterozygous transition mutation 3418C → T resulting in nonsense mutation R1140X. (D) Left panel shows the normal sequence from *FLG* repeat 4 corresponding to codons 1473–1476. Right panel shows a heterozygous transition mutation 4420C → T resulting in nonsense mutation R1474X. (E) Left panel shows the normal sequence from *FLG* repeat 5 corresponding to codons 1789–1792. Right panel shows a heterozygous transition mutation 5368C → T resulting in nonsense mutation

Q1790X. (F) Left panel shows the normal sequence from *FLG* repeat 7 corresponding to codons 2416–2419. Right panel shows a heterozygous transition mutation 7249C → T resulting in nonsense mutation Q2417X. (G) Left panel shows the normal sequence from *FLG* repeat 7 corresponding to codons 2421–2424. Right panel shows a heterozygous transition mutation 7264G → T resulting in nonsense mutation E2422X. (H) Left panel shows the normal sequence from *FLG* repeat 7 corresponding to codons 2705–2708. Right panel shows a heterozygous transition mutation 8117C → G resulting in nonsense mutation S2706X. (I) Left panel shows the normal sequence from *FLG* repeat 10.2 corresponding to codons 4305–4308. Right panel shows a heterozygous transition mutation 12916C → T resulting in nonsense mutation R4306X. (J) Left panel shows the normal sequence from *FLG* partial repeat (repeat 11) corresponding to codons 4670–4673. Middle panel shows a heterozygous transition mutation 14011A → T resulting in nonsense mutation K4671X. Right panel shows a homozygous transition mutation 14011A → T resulting in nonsense mutation K4671X.

Singaporean Chinese and Chinese shared the similar ancestry. However, mutation 3321delA, which was first identified in Japanese and also commonly seen in our study, was not found in Singaporean Chinese IV patients (27) despite the

fact that they share the similar ancestry. Except for the commonly seen mutation K4671X other nine novel *FLG* mutations were rare in our patients with AD and not found in healthy controls as well, indicating that they were very

Table 2 The prevalence of mutations in filaggrin (*FLG*) in patients with atopic dermatitis (AD)

Novel <i>FLG</i> Mutation	Filaggrin repeat number (position of a stop codon)	<i>n</i> (%) AD	Reported <i>FLG</i> Mutation	Filaggrin repeat number (position of a stop codon)	<i>n</i> (%) AD
R826X	2 (p.826)	2 (0.8)	441delA	S100 domain (p.193)	1 (0.4)
3222del4	2 (p.1120)	2 (0.8)	R501X	1 (p.501)	1 (0.4)
R1140X	3 (p.1140)	1 (0.4)	3321delA	2 (p.1121)	38 (14.6)
4271delAA	3 (p.1448)	2 (0.8)	R1474X	4 (p.1474)	1 (0.4)
Q1790X	5 (p.1790)	3 (1.1)	Q2417X	7 (p.2417)	7 (2.7)
5757del4	5 (p.2093)	1 (0.4)	E2422X	7 (p.2422)	3 (1.1)
6834del5	6 (p.2318)	1 (0.4)	7945delA	7 (p.2742)	1 (0.4)
6950del8	6 (p.2317)	3 (1.1)	R4306X	10.2 (p.4306)	2 (0.8)
S2706X	7 (p.2706)	1 (0.4)			
K4671X	Imperfect filaggrin repeat (p.4671)	24 (9.2)			

Table 3 Analysis of association between the filaggrin (*FLG*) mutations and atopic dermatitis (AD) and AD-associated phenotypes

	Combined <i>FLG</i> genotypes				3321delA				K4671X			
	AA	Aa	aa	<i>P</i> -value	AA	Aa	aa	<i>P</i> -value	AA	Aa	aa	<i>P</i> -value
AD	179	62	20	0.000*	223	35	3	0.000*	237	20	4	0.004*
Controls	92	0	0		92	0	0		92	0	0	
AD with asthma	89	25	7	0.265	111	9	1	0.016**	109	9	3	0.572
AD without asthma	90	37	13		112	26	2		128	11	1	
AD with AR	81	24	7	0.553	98	13	1	0.777	103	7	2	0.700
AD without AR	98	38	13		125	22	2		134	13	2	
AD with elevated IgE	112	38	12	0.536	140	19	3	0.215	148	10	4	0.235
AD with normal IgE	39	16	7		50	12	0		55	7	0	
AD with elevated ECP	108	48	14	0.052	142	26	2	0.064	150	17	3	1.000
AD with normal ECP	4	0	2		4	1	1		6	0	0	
Allergy test												
fx5E +	72	41	7	0.012**	98	22	0	0.092	106	12	2	0.379
–	48	11	9		58	8	2		63	3	2	
hx2 +	83	33	9	0.848	107	16	2	0.352	112	10	3	0.728
–	38	18	5		49	12	0		56	5	0	

AA refers to wild-type/wild-type *FLG* genotype for 3321delA and K4671X and all the other *FLG* mutation variants; Aa refers to heterozygous genotype for 3321delA or K4671X or any other *FLG* mutation variants, and aa refers to homozygous genotype for 3321delA or K4671X or any other *FLG* mutation variants or compound heterozygous genotype. AR, allergic rhinitis; ECP, eosinophilic cationic protein.

* $P < 0.01$; ** $P < 0.05$.

rare or family specific in Chinese population. Our results further proved that *FLG* mutation spectrum of Chinese population dramatically differed from that of European population. Thus, the population-specific *FLG* mutation spectrum will give us a clue to make pointed screening of *FLG* mutations in different populations.

Filaggrin mutations were present in 82 (31.4%) cases of patients with AD and none of the healthy controls, indicating a strong association between *FLG* mutations (compound genotypes) and AD in our study (OR 1.458, 95%CI 1.343–1.583, $P < 0.001$, Table 3). We confirmed that *FLG* mutations were significant predisposing factors for AD in Chinese as well as in European and in Japan. As mentioned earlier, the healthy controls were without history of AD, any atopic manifestations and IV, i.e. this is a hypernormal control

rather than a general population cohort. These results further confirmed the powerful role of *FLG* mutations in the pathogenesis of AD.

We also found that individuals carrying *FLG* mutations had a much earlier age of onset than those not carrying any *FLG* mutations, indicating the effect of *FLG* mutations on the age of onset for AD. *FLG* mutations (compound genotypes) were also strong risk factors for a subgroup of AD patients with food sensitization ($P = 0.012$, Table 3). Weidinger et al. (28) also found that *FLG* mutations predisposed for AD with allergic sensitization. Schuttelaar et al. (29) confirmed that early-life cat exposure enhances the effect of a *FLG* mutation on the development of eczema and sensitization. As we have known, disruption of normal epidermal barrier function is also considered as a key event in allergic

sensitization. Impaired epidermal barrier allows allergens penetrate into the skin and makes them exposure to antigen-presenting cells, leading to allergic sensitization. Filaggrin is expressed in epidermis and also in oral and nasal mucosa, in which it is also presumed to contribute to epithelial barrier function. Our results suggested that skin barrier defects because of *FLG* mutations might play a crucial role in the pathogenesis of food sensitization.

Several large cohort studies have confirmed that *FLG* mutations were strong risk factors for asthma and allergic rhinitis involved in the atopic march (7, 11), increased asthma severity (13) and the severity of AD (9). A meta-analysis by Rodríguez et al. (30) suggested that *FLG* mutations conferred strong risk for the compound phenotype asthma plus eczema but not for asthma in the absence of eczema. However, we did not replicate any significant association between *FLG* mutations (compound genotypes) and other allergic phenotypes, namely asthma, allergic rhinitis and elevated total serum IgE levels as well as elevated serum ECP levels ($P > 0.05$, Table 3). The reason for the negative association between *FLG* mutations and atopies in our study is not clear, and we speculated that it may be caused by sample bias. The majority of our patients were well below the median age at onset of asthma and allergic rhinitis. A follow-up study of these patients with AD will allow us further assessing the relationship between *FLG* mutations and AD-associated asthma and allergic rhinitis.

We did not observe an association between *FLG* mutations and the severity of AD (evaluated by the SCORAD) in line with two recent studies (22, 31). In another study, *FLG* mutations were weakly associated with disease severity (9). It is noteworthy that the SCORing Atopic Dermatitis index was used for assessing the severity of disease in these different studies. As we have known, the severity of AD varies greatly depending on distribution and intensity of skin lesions, the course of the disease, aggravating factors and concomitant complications such as superinfection and so on. Although *FLG* mutations resulting in disturbance of epidermal barrier function seems to be one of the central aspects in the pathophysiology of AD, *FLG* mutations cannot influence the severity of AD, i.e. the SCORAD index by itself.

The most common mutation 3321delA was also significantly associated with AD (OR 1.413, 95% CI 1.316–1.516, $P < 0.001$, Table 3). Interestingly, a significant association between mutation 3321delA and AD with asthma (OR 0.360, 95% CI 0.167–0.777, $P = 0.016$, Table 3) was observed, and a low OR value indicated its protective effect for asthma occurring in the context of AD, which was inconsistent with the findings from Ma et al. (15). Notably, in their study, the average age of patients with AD (30.7 ± 12.5 years) was older than that of our patients with AD (7.11 ± 8.39 years). So, we still speculated that the contrary result was also attributable to the sample bias mentioned earlier. We also found that exclusion of those subjects under 3 years of age made the association not significant ($P > 0.05$, data not shown). Therefore, further *FLG* mutations analysis in a group of older Chinese patients with AD will allow us to

evaluate the exact effect of 3321delA mutation on AD-associated asthma. In addition, this study could not detect any significant association between 3321delA mutation and allergic rhinitis, elevated total serum IgE levels, elevated serum ECP levels and allergic sensitization ($P > 0.05$, Table 3).

The other common mutation K4671X also significantly associated with AD (OR 1.388, 95% CI 1.298–1.485, $P = 0.004$, Table 3). Similarly, mutation K4671X did not associate with atopies, including asthma, allergic rhinitis, elevated total serum IgE levels, elevated serum ECP levels and allergic sensitization ($P > 0.05$, Table 3). Mutation K4671X was located in the last partial filaggrin repeat ('repeat 11') close to the C-terminal domain of profilaggrin, which comprises 157 amino acids (16). The precise function of the C-terminal domain remains unclear, but its expression seems to be prerequisite for monomeric filaggrin processing (18). So, we could predict that mutation K4671X, which was close to the C-terminal domain, might lead to complete loss of processed filaggrin expression.

The eighteen *FLG* mutations identified in this study were located in several filaggrin repeats, mainly in filaggrin repeat 2, 3, 5, 6 and 7. It is unclear whether these *FLG* mutations act equally on the processed filaggrin expression because biopsy material from these patients who carrying *FLG* mutations was not available for study. Further immunohistochemical analysis of the filaggrin expression of the biopsy specimen of these patients carrying different heterozygous or homozygous or compound heterozygous *FLG* mutations will identify their effect on disease.

The frequency of *FLG* mutations in our AD cohort was 31.4%, higher than that in the Japanese (over 20%) (24) but lower than that in the European populations (over 40%) (14, 18). It seems that individuals carrying *FLG* null mutations represent a significant group of the at-risk population for AD and AD-associated atopic manifestations. If early intervention for the maintenance and repair of the epidermal barrier is effective for preventing the subsequent development of the 'atopic march', we may consider the screening for *FLG* mutations to be a noninvasive test for screening or forecast of AD in an at-risk population in the clinical work. It would be necessary for the researchers to continue to emphasize the clinical importance of *FLG* mutations.

In conclusion, we have uncovered ten novel *FLG* mutations in Han Chinese population for the first time and put the total number of *FLG* mutations over forty. *FLG* mutations strongly associate with AD and also predispose to food sensitization in Han Chinese population. These data emphasize the essential role of *FLG* mutations in the development of AD in Chinese.

Acknowledgments

We thank all the patients for their participation and Prof. Long Yu for his technical support. We also thank the nurses, Weiqin Yang, Chunyan Mao, Yufang Li, Beibei Zhang, Xiaojuan Pan and Yumin Sun for their support to this study. This study was funded by a grant from Science and Technology Commission of Shanghai Municipality (08JC1415900).

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