

Research letter

Filaggrin gene mutations may influence the persistence of food allergies in Japanese primary school children

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DEAR EDITOR, Mutations in *FLG*, encoding filaggrin, are the underlying cause of ichthyosis vulgaris and are an important predisposing factor for atopic dermatitis (AD).¹ In 2011, *FLG* mutations were reported to increase the risk of peanut allergy,² and they have been proven to increase the risk of other food sensitizations and allergies.^{3,4} In this study, we comprehensively screened 411 children in Japan for 10 *FLG* mutations specific to the Japanese population and investigated whether *FLG* mutations influence the persistence of food allergies (FAs).

To investigate the association of *FLG* mutations with allergies, particularly FAs, in a general paediatric population, we studied pupils at a primary school in Japan. Of the 411 pupils, 410 were measured for total IgE and six antigen-specific IgEs.⁵ Of these 410 pupils, the present study covers the 375 who answered our questionnaire⁵ only once each. We used a portion of the questionnaire survey data (Table S1; See Supporting Information) and the IgE data to survey the allergic conditions of elementary school-age children. The FA, AD, asthma and allergic rhinitis outcomes were parent reported and physician diagnosed. Details of samples and methods are also available in Table S2 (See Supporting Information). The

local institutional review boards approved this study, and all guardians of the children provided written informed consent.

In the present *FLG* mutation screening, we studied the 10 *FLG* mutations specific to the Japanese population (Table S3; See Supporting Information). All are loss-of-function mutations. Genotyping of the *FLG* mutations was performed with the TaqMan probe genotyping assay as in our previous study.⁶

Of the 375 participants with outcome data available, 28 (7.5%) were heterozygous for one of the 10 *FLG* mutations. According to the present data obtained from the 374 pupils through the clinical questionnaire, 14 individuals reported current FAs (3.7%). Among the total of 374 pupils, four of the 28 individuals with *FLG* mutations (14%) had current FA and 10 of the 346 individuals without *FLG* mutations (2.9%) had current FA. Thus, *FLG* mutations were significantly associated with current FAs [odds ratio (OR) 5.60, 95% confidence interval (CI) 1.64–19.2, $P = 0.0023$] (Table 1). Furthermore, *FLG* mutations were significantly associated with current FAs that occurred in combination with current or past AD (OR 5.96, 95% CI 1.43–24.8, $P = 0.0059$) (Table 1). In contrast, the association of *FLG* mutations with current and past FAs was not significant (OR 1.69, 95% CI 0.55–5.20, $P = 0.35$).

The group with past FA consisted of primary school students who had a history of FAs but who reported having no current FAs in the present study. All the participants answered the questionnaire only once, and positive past FA means that a participant answered that he or she once had FA at any time point prior to his or her participation in the present study

Table 1 Prevalence of allergic disease among all of the participants ($n = 375$) and the subgroups of participants with the combined genotype (one or more *FLG* mutations) ($n = 28$) and without the combined genotype (no *FLG* mutations) ($n = 347$)

History ^a	All individuals, % (n/N)	Individuals with <i>FLG</i> mutations, % (n/N)	Individuals without <i>FLG</i> mutations, % (n/N)	OR (95% CI)	P-value
Current FA	3.7 (14/374)	14 (4/28)	2.9 (10/346)	5.60 (1.64–19.2)	0.0023
Past FA	5.6 (21/374)	0 (0/28)	6.1 (21/346)	NA	NA
Current FA with current or past AD	2.7 (10/374)	11 (3/28)	2.0 (7/346)	5.96 (1.43–24.8)	0.0059
Current FA without current or past AD	1.1 (4/374)	4 (1/28)	0.9 (3/346)	4.23 (0.43–40.1)	0.18
Current and past FA	9.4 (35/374)	14 (4/28)	9.0 (31/346)	1.69 (0.55–5.20)	0.35
Current AD	10.1 (38/375)	14 (4/28)	9.8 (34/347)	1.53 (0.50–4.68)	0.45
Current allergic rhinitis	38.1 (143/375)	36 (10/28)	38.3 (133/347)	0.89 (0.40–2.00)	0.78
Current asthma	9.7 (37/375)	11 (3/28)	9.8 (34/347)	1.10 (0.32–3.85)	0.88

FA, food allergy; AD, atopic dermatitis; OR, odds ratio; CI, confidence interval; NA not applicable. ^aBoth current allergy and past allergy history are from answers to the questionnaire obtained at only one time point for each participant. In addition, past FA means a positive history of FA at any time in the past, and the participants with past FA did not have a uniform time frame for their allergy history. The intervals from the past FA to current absence of FA are different among participants in the past FA group.

(Table 1). We had 21 participants in the past FA group. Interestingly, none of them had any FLG mutations. From this fact, we speculate that FLG mutation carriers with past FAs rarely grow out of their FAs. If this speculation is true, the present results might suggest that FLG mutations are associated with the persistence of FA from the infantile period to the primary school years, although this speculation is not based on a statistically significant sample.

The reason there were no FLG mutation-carrying individuals with past FAs is probably because it takes time for the children to grow out of their FAs, and for nuts and peanuts, they usually do not. In other words, patients with FA transiently seen in the infantile period without FLG mutations tend to remit spontaneously. As the patients with FLG mutation have continuous incidences of percutaneous sensitization, FAs in the individuals with FLG mutations remain at primary school age. Thus, FLG mutations might be associated with the persistence of FAs. Indeed, FLG mutations were reported to have a significant effect on the risk of FA in later childhood (age 10 years), but an insignificant effect at the ages of 1, 2 and 4 years.³ It has been reported that FLG mutation is associated with IgE sensitization to peanuts, but not to other food allergens.⁷

We also investigated the association between FLG mutations and allergy to each allergen. However, children allergic to each allergen were too few to be significant (See Supporting Information).

In conclusion, the present study suggests that FLG mutations are a significant predisposing factor for FAs in primary school students and may influence the persistence of FAs from infancy to the primary school years. In a future study, the association between FA and FLG mutations needs to be determined in patients with FA who are diagnosed by food-challenge tests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1 Summary of questionnaires for status definition of food allergy, atopic dermatitis, allergic rhinitis and asthma in the participants of the present study.

Table S2 Characteristics of the study population.

Table S3 FLG genotypes in the present cross-sectional study of primary school students.

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Supplementary Information

Supplementary Samples and Methods

To investigate the association of *FLG* mutations with allergies, particularly food allergies, in a general pediatric population, we studied pupils at a primary school in Chiba city, a suburb of Tokyo, Japan. Of the 411 pupils, 410 pupils were measured for total IgE and 6 antigen-specific IgEs.¹ Of these 410 pupils, the present study covers the 374 pupils (194 males and 180 females, primary school grades 1 through 6 (ages 6 through 12)) who answered our questionnaire.¹ We used a portion of the questionnaire survey data to determine the allergic conditions, in addition to blood test data including 6 serum food- and aeroallergen-specific IgEs. Our study was originally set up to survey allergic diseases and obesity, and their background factors, in elementary school children.

In this study, we used the questionnaire data to determine the presence/absence of present and past food allergy illness, atopic dermatitis, allergic rhinitis and asthma for

each participant (Supplementary Table 1).

Present allergy illness and previous allergy illness was diagnosed based on the questionnaire in supplementary Table 1. The positive current illness group consisted of participants who responded “Yes” to Q1, “Yes or Suspected” to Q3, and “Yes” to Q4. The positive past illness group consisted of the participants who answered “Yes” to Q1, “Yes or Suspected” to Q3, and “No” to Q4, or the participants who answered “No” to Q1, “Yes” to Q3, and “No” to Q4. The other participants were determined as not having food allergies.

For atopic dermatitis, the status of present illness or previous illness was diagnosed based on the questionnaire in supplementary Table 1. The positive current illness group consisted of the participants who answered “Yes” to Q2. The positive past illness group consisted of the participants who answered “Yes” to Q1 and “No” to Q2. The other participants were defined as healthy with respect to atopic dermatitis.

For allergic rhinitis, the status of present illness or previous illness was diagnosed by modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire in supplementary Table 1.² The positive current illness group consisted of the participants who answered “Yes” to Q1, “Yes” to Q2, and “Yes” to Q3. The positive past illness group consisted of the participants who answered “Yes” to Q1, “No” to Q2, and “Yes” to Q3. The other participants were defined as healthy with respect to rhinitis.

For asthma, the status of present illness or previous illness of participants was diagnosed by modified ISAAC questionnaire in supplementary Table 1.² The positive current illness group consisted of participants who answered “Yes” to Q1, “Yes” to Q2, “more than once” to Q3, and “Yes or Suspected” to Q4. The positive past illness group consisted of the participants who answered “Yes” to Q1, “No” to Q2, “No” to Q3, and “Yes” to Q4. The other participants were defined as healthy with respect to asthma.

Characteristics of the study population in the present study are shown in Supplementary Table 2.

We calculated the power to detect association of *FLG* mutations and the disease conditions with the aid of the IBM SPSS SamplePower program ver. 3.0 (IBM Corporation, USA). Given the prevalence of the diseases and the frequency of *FLG* mutations in our samples, the power ($\alpha=0.05$) to detect an association between current AD and *FLG* mutations was estimated as 65.6% (if the OR of the mutation=4.0) or 81.4% (if the OR of the mutation=5.0). We also estimated that 80% power ($\alpha=0.05$) could be achieved if the ORs of *FLG* mutation were as follows: 6.38 (current asthma), 4.85 (current AD), 2.85 (current AR), 10.5 (current FA).

Supplementary Results and Discussion

***FLG* genotypes of primary school students in this study**

Of the 411 participants, 29 individuals were heterozygous for 1 of the 10 *FLG* mutations and no individuals were compound heterozygous or homozygous for the mutations. The distribution of genotypes is shown in Supplementary Table 2. The mutant allele frequency was 0.035 and the carrier rate was 0.071. The prevalence rate of

the combined genotype (one or more of the 10 *FLG* mutations) was similar to that of a 2014 study in the Japanese population³ and was lower than that of our 2014 study in the Japanese population for 10 *FLG* mutations in Hokkaido, Japan.⁴ The present study was performed in a suburban area near a big city, and we expected much more mobility of people in the present subject group. Thus, the prevalence observed in the present study might approximate the average Japanese prevalence of *FLG* mutations.

The association between *FLG* mutations and sensitization and allergy to each food allergen

We investigated the association between *FLG* mutations and allergy to each allergen. However, there were too few children allergic to each allergen to be significant. The allergies were as follows: eggs (24 children), fruits and vegetables (9 children), shellfish (5 children), milk (5 children), egg products (5 children), and dairy products (4 children). (Supplementary Table 1)

Serum IgE specific for orchard grass, *Alternaria* fungi, mites, cedar pollen, cats, and

ovalbumin was measured. We set two alternative definitions, defining positive as “Class 1 or higher”, or “Class 2 or higher”. Then, we investigated the association between *FLG* mutations and sensitization to each allergen using the two alternative definitions. However, no significant association with *FLG* mutations was detected using either definition.

The association between *FLG* mutations and total serum IgE level

We investigated the association between *FLG* mutations and total serum IgE levels. We obtained 410 measured values from the 411 participants in the present *FLG* mutation analysis. Of these 410 participants, average measured values of total serum IgE concentrations of 181.24 IU/mL and 253.45 IU/mL were obtained in *FLG* mutation-carrying individuals (n=29) and non-*FLG* mutation-carrying individuals (n=381), respectively. The total serum IgE levels showed a significant difference (p=0.0350) between *FLG* mutation-carrying individuals and non-*FLG* mutation-carrying individuals.

The association between FAs and AD

In the present study, the association of FAs with AD was significant for current AD patients (odds ratio=14.67 [95% CI: 4.77-45.07, $p<0.001$]) and for current and past AD patients (odds ratio=11.14 [95% CI: 3.39-36.60, $p<0.001$]). These results suggest that FAs are associated with AD.

The association of *FLG* mutations with AD

Concerning AD, 4 individuals out of the 28 with *FLG* mutations (14.3%) were currently affected with AD, and 34 pupils out of the 347 individuals without *FLG* mutations (9.7%) were currently affected with AD. Thus, current AD was not significantly associated with *FLG* mutations (odds ratio=1.53 [95% CI: 0.51-4.68, $p=0.449$]) (Table 1). These results suggest that the strength of association between *FLG* mutations and AD might be weaker than that between *FLG* mutations and FAs in the primary school years. Alternatively, the limitation of statistical power (see Supplementary Samples and Methods) might be a reason for the failure to detect a significant association.

The association of *FLG* mutations with asthma and allergic rhinitis

In our study, neither asthma nor allergic rhinitis was significantly associated with *FLG* mutations. In previous reports in Japan, some studies demonstrated an association between *FLG* mutations and AD, asthma⁵ and allergic rhinitis⁴. The present results, i.e., the non-significant association of *FLG* mutations with AD, asthma, or allergic rhinitis, may be a result of the small sizes of the subject groups.

Supplementary References

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asthma. *J Invest Dermatol* 2010; **130**: 2834-6.

Supplementary Table 1. Summary of questionnaires for status definition of food allergy, atopic dermatitis, allergic rhinitis, and asthma in the participants of the present study

Food allergies

Q1. Has your child had a rash or felt sick within 2 hours after eating a particular food (except for food poisoning)? [Yes, No]

Q2. If yes, then what was it that your child ate? [eggs, egg products, milk, dairy products, soybeans, wheat, peanuts, buckwheat, shellfish, fish, salmon roe, fruits or vegetables, other]

Q3. Has your child ever been diagnosed with food allergy by a physician? [Yes, Suspected, No]

Q4. Does your child have a food allergy now? [Yes, No]

Atopic dermatitis

Q1. Has your child ever been diagnosed with atopic dermatitis by a physician? [Yes, No]

Q2. Does your child currently have atopic dermatitis, as diagnosed by a physician? [Yes, No]

Allergic rhinitis

Q1. Has your child ever had a problem with sneezing, or a runny or blocked nose when he/she did not have a cold or the flu? [Yes, No]

Q2. In the past 12 months, has your child had a problem with sneezing, or a runny or blocked nose when he/she did not have a cold or the flu? [Yes, No]

Q3. Has your child ever been diagnosed with hay fever by a physician? [Yes, No]

Asthma

Q1. Has your/ child ever had wheezing or whistling in the chest at any time in the past? [Yes, No]

Q2. Has your child had wheezing or whistling in the chest in the past 12 months? [Yes, No]

Q3. How many attacks of wheezing has your child had in the past 12 months? [None, 1

to 3, 4 to 12, More than 12]

Q4. Has your child ever been diagnosed with asthma by a physician? [Yes, Suspected, No]

Supplementary Table 2. Characteristics of the study population

Total no. of participants	375
Age (mo)	
Mean $\pm$ SD	111.1 $\pm$ 19.9
Range	76-147
Sex ratio (male:female)	1.07:1.00
Total IgE (IU/mL), mean $\pm$ SD	
Male	253.5 $\pm$ 340.9
Female	242.2 $\pm$ 470.3
Prevalence of allergic disorders (%)	
Asthma	
Male	12.9
Female	6.6
Atopic dermatitis	
Male	11.3
Female	8.8
Allergic rhinitis	
Male	43.8
Female	32.0
Food allergy	
Male	3.1
Female	4.4

Supplementary Table 3. *FLG* genotypes in the present cross-sectional study of primary school students

Genotype	p.Arg501X	c.3321delA	p.Ser1695X	p.Gln1701X	p.Ser2554X	p.Ser2889X	p.Ser3296X	p.Lys4022X	p.Q1790X	c.441-442delAG	Combined
AA	411	401	411	411	409	404	409	403	411	411	382
Aa	0	10	0	0	2	7	2	8	0	0	29
aa	0	0	0	0	0	0	0	0	0	0	0
Total	411	411	411	411	411	411	411	411	411	411	411
Allele freq. (%)	0.0	1.22	0.0	0.0	0.24	0.85	0.24	0.97	0.0	0.0	3.53