



Letter

Food sensitization in Japanese infants is associated with a common *Filaggrin* variant

The discovery of the association between loss-of-function mutations in *filaggrin* (*FLG*) and atopic eczema¹ proved that atopic eczema is not only an immunological disease but also a skin barrier disease. Eight *FLG* null mutations were found to have accumulated in 27% of atopic eczema patients and in 4% of the control population in Japan.² Relationships of *FLG* null mutations with other allergic diseases such as bronchial asthma and allergic rhinitis also have been reported.³ However, the role of common variants of *FLG* in atopic eczema⁴ and other allergic diseases is poorly understood.

Filaggrin dysfunction has been implied to facilitate food sensitization. In a loss-of-function *FLG* mutation mouse model, topical application of an allergen led to allergen sensitization.⁵ More recently, an *FLG* null mutation was shown to be a significant risk factor in peanut allergy⁶ and food sensitization among infants.⁸ In the current study, we focused on the relationship between common *FLG* variants and food sensitization in a cross-sectional study on Japanese infants.

Japanese infants ($n = 116$) aged 9 to 14 months who visited our pediatric clinic and hospitals for potential food allergy were enrolled in the current study (eTable 1). Written informed consent was obtained from their parents. A pediatrician diagnosed 58 (50%) of the 116 infants with atopic eczema on the basis of the criteria of Hanifin and Rajka.⁷ Immunoglobulin E (IgE) antibodies specific to 5 of the most common food allergens in Japanese infants (egg white, cow's milk, wheat, soybean, and peanut) were measured by the ImmunoCAP test (Phadia AB, Uppsala, Sweden). We assessed the magnitude of food sensitization by using a number of positive (≥ 0.35 IU/mL) food-specific IgEs. The study was approved by the Local Research Ethics Committee and was conducted according to the Declaration of Helsinki Principles.

Genomic DNA samples were extracted from peripheral whole blood using the standard protocol. Known loss-of-function *FLG* mutations (R501X, 3321delA, S1695X, Q1701X, S2554X, S2889X, S3296X, K4022X) and single nucleotide polymorphisms (SNPs) near *FLG* (rs1933064, rs12730241) were genotyped using the Custom TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, California) according to manufacturer instructions. *FLG* alleles were either “wild-type” (all 8 loci were homozygous wild-type), or “non-wild-type” for all combined null mutations.

We used Fisher's exact test to assess the association between 2 dichotomous variables, and Wilcoxon rank sum test or Kruskal–Wallis test for ordinal dependent variables. A proportional odds model—a logistic model for ordinal dependent variables—was

used to adjust for atopic eczema and combined *FLG* null mutation. $P < .05$ was considered significant. Statistical analyses were performed using JMP 8.0.2 (SAS Institute, Cary, North Carolina).

The associations of *FLG* genotypes with the number of positive food-specific IgEs are summarized in Table 1. A minor allele of rs1933064 (G allele) was negatively related to the magnitude of food sensitization ($P = .029$). Although atopic eczema was related to food sensitization ($P < .0001$), the association between rs1933064 and food sensitization was observed even after adjusting for concomitant atopic eczema and combined *FLG* null mutation ($P = .0055$). We did not find a relationship for combined *FLG* null mutation with food sensitization ($P = .45$) or atopic eczema ($P = .60$). However, if we assessed food sensitization by the status of nonsensitization or any sensitization, combined *FLG* null mutation was also associated with food sensitization ($P = .039$). We also found a negative relationship between a minor allele of rs1933064 and higher ImmunoCAP classes (eTable 2) for egg white ($P = .025$) and cow's milk ($P = .019$).

The current study revealed a novel finding. A common variant of *FLG* plays an important role in food sensitization, irrespective of concomitant null mutations of *FLG*. Because rs1933064 lies 3.9 kbp upstream of *FLG*, it might affect promoter activity of *FLG*, thereby modifying skin barrier function. How a minor allele of the SNP is protective for food sensitization independently of atopic eczema remains unknown. However, another study on a European American population⁹ also reported an SNP in the promoter region (rs1933063) associated with reduced risk of atopic dermatitis eczema herpeticum, lower total IgE levels, and mild symptoms of atopic eczema. Unexpectedly, we did not detect any relationship between *FLG* genotype and atopic eczema that was

Table 1

Filaggrin genotypes and the number of positive food-specific IgEs ($n = 116$)

No. of positive food-specific IgEs	0	1	2	3	4	5	P-value
Combined null mutation							.45
Wild-type	21	25	17	9	13	14	
Non-wild-type	0	6	6	1	2	2	
rs12730241							.19
AA	11	12	6	6	9	12	
AG	7	13	12	4	5	3	
GG	3	6	5	0	1	1	
rs1933064							.029 ^b (.0055) ^{a,b}
AA	13	20	14	9	14	14	
AG	6	10	7	1	1	2	
GG	2	1	2	0	0	0	

Wild-type, all 8 loci (R501X, 3321delA, S1695X, Q1701X, S2554X, S2889X, S3296X, K4022X) were homogenous wild-type; Non-wild-type, at least 1 mutation in the 8 loci. AA/AG/GG, allelic variants at rs12730241 and rs1933064.

^aAdjusted for atopic eczema and combined null mutation.

^b $P < .05$.

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reported in 1-year-old infants.⁸ Our allergy-biased study population may have made it difficult to detect the relationship. Further studies with a larger population and other allergic diseases would be needed.

To our knowledge, this study is the first to show the association between a common variant of *FLG* and food sensitization, and it provides important information on the significance of skin barrier function in the cause of food allergy.

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Supplementary Data

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.anai.2013.02.003>

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eTable 1Population characteristics (*n* = 116)

Characteristics	Values	
Median age in months (range)	12	(9–14)
Male sex	57.8%	(67/116)
Atopic eczema	50.0%	(58/116)
Ichthyosis vulgaris	0%	(0/116)
Laboratory data: Median (quartile)		
Eosinophil granulocyte (/μL)	262.5	(106.8–480)
Total IgE (IU/mL)	60.5	(20.0–194.3)
TARC (pg/mL) ^a	995	(565–1,813)
<i>Filaggrin</i> null mutations		
R501X	0%	(0/116)
3321delA	4.3%	(5/116)
S1695X	0%	(0/116)
Q1701X	0%	(0/116)
S2554X	1.7%	(2/116)
S2889X	6.0%	(7/116)
S3296X	0%	(0/116)
K4022X	3.4%	(4/116)
Combined	14.7%	(17/116)

TARC, thymus and activation-regulated chemokine; *Filaggrin* null mutations, having ≥ 1 null alleles.^aOne missing value.**eTable 2**rs1933064 genotype (AA/AG/GG) and ImmunoCAP classes of each food (*n* = 116)

ImmunoCAP class	0	1	2	3	4	5	6	P-value
rs1933064								
	Egg White							.025 ^a
AA	13	2	15	30	13	8	3	(.0068) ^{a,b}
AG	7	1	6	11	0	2	0	
GG	2	0	2	1	0	0	0	
rs1933064								
	Cow's milk							.019 ^a
AA	37	6	15	14	7	4	1	(.0063) ^{a,b}
AG	19	2	2	2	1	1	0	
GG	4	1	0	0	0	0	0	
rs1933064								
	Wheat							.11
AA	49	4	17	8	3	2	1	
AG	21	2	2	1	1	0	0	
GG	4	0	1	0	0	0	0	
rs1933064								
	Soybean							.06
AA	55	7	12	7	3	0	0	
AG	23	1	1	2	0	0	0	
GG	5	0	0	0	0	0	0	
rs1933064								
	Peanut							.19
AA	65	3	5	7	3	1	0	
AG	24	1	2	0	0	0	0	
GG	5	0	0	0	0	0	0	

^a*P* < .05.^bAdjusted for atopic eczema and combined *FLG* null mutation.