

BRIEF COMMUNICATION

Loss-of-function variants of the filaggrin gene are associated with clinical reactivity to foods

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Abstract

The aim of this study was to assess the genetic association of *Filaggrin* loss-of-function (*FLG* LOF) genetic variants with food allergy, and to investigate the added value of this test in diagnosing food allergy. Clinical reactivity to foods was diagnosed by the gold standard, the double-blind, placebo-controlled food challenge. Of 155 children, 33 (21.3%) children had at least one *FLG* LOF variant, and of these, 29 (87.9%) were clinically reactive to at least one food, compared to 73 of 122 children (59.8%) carrying wild-type alleles. The odds ratio for having at least one *FLG* LOF variant and clinical reactivity to at least one food was 4.9 (CI = 1.6–14.7, $P = 0.005$), corresponding to a relative risk of 1.5, compared to carriers of wild-type alleles. Prediction of food allergy improved when *FLG* LOF variants were included in the model. Therefore, genetic markers may be useful as an addition to clinical assessment in the diagnosis of food allergy.

Filaggrin gene loss-of-function (*FLG* LOF) variants, located in the epidermal differentiation complex, are strong risk factors for eczema (1, 2) and may play a role in food allergy, perhaps by allowing food allergens to pass through the compromised skin barrier more easily. Whether this results in sensitization, clinical reactivity, or both with respect to foods is recently a point of debate in the literature (3–5).

Methods

All children undergoing double-blind, placebo-controlled food challenge (DBPCFC) at the food challenge unit (FCU) of the University Medical Center Groningen (UMCG) between 2005 and 2009 and their parents were asked for writ-

ten informed consent and included in this cross-sectional study (METc 2004-146).

DBPCFCs were performed as previously described (6). They were part of regular tertiary pediatric allergy care in children with a history consistent with a type 1 reaction after ingestion of a food. Children were classified by the following definitions:

- Clinically reactive to foods if they had at least one positive DBPCFC to at least one food
- Not clinically reactive if they had only negative DBPCFCs.

Buccal mucosa samples were collected from both parents and the child, and DNA was extracted by the genetics lab of the UMCG and stored at -80°C . Four gene variants were genotyped (*R501X*, *S3247X*, *2282Del4*, and

R2447X) by competitive allele-specific PCR using KASPar genotyping chemistry, under contract by LGC Genomics (LGC, Teddington, UK). Food-specific IgE (sIgE) was measured by CAP-FEIA (ImmunoDiagnostics, Uppsala, Sweden), and a value above 0.35 kU/l was defined as being positive.

Linkage disequilibrium (LD), call rate, and Hardy–Weinberg equilibrium were tested by Haploview (7). For family-based analysis and testing for Mendelian errors, we used FBAT software version 2.0.4 using the additive model (8). Associations were determined based on chi-square tests and logistic regression analysis using SPSS 20.0 (IBM, Chicago, IL, USA). To convert odds ratio (OR) to relative risk (RR), we used the formula: $RR = OR / 1 + \text{control event rate} \times (OR - 1)$. Atopic comorbidities (asthma, eczema, and rhinitis) and sIgE values were considered to be confounders when they changed the beta coefficient of the effect of the genetic variable on test outcome in a logistic regression by 10% or more. For all tests, a two-tailed significance level of $P < 0.05$ was used.

Results

In total, 173 trios enrolled in the study. Of these, 18 were excluded due to individual call rates $<50\%$ ($n = 4$), non-Caucasian ethnicity ($n = 5$), equivocal result of the DBPCFC ($n = 5$), or Mendelian errors which indicated nonpaternity ($n = 4$). Of all gene variants, Hardy–Weinberg P -values were above 0.0125 and call rates above 95%; therefore, none of them were excluded from analysis. None of the gene variants were in LD with one another ($r^2 < 0.01$).

Of the 155 children (mean age 7.5 years, SD 57.4 months), 102 (65.8%) were found to be clinically reactive to at least one food. The prevalence of sensitization was 79.7% ($n = 118$), and 31 children were asymptotically sensitized to the food(s) tested. The majority ($n = 131$, 84.5%) reported eczema by history (Table 1).

Of the 155 children, 33 (21.3%) children had at least one *FLG* LOF variant, and of these, 29 (87.9%) were clinically reactive to at least one food. The OR for having at least one *FLG* LOF variant and being clinically reactive was 4.9 (95% CI = 1.6–14.7, $P < 0.1$) which corresponds to a RR of 1.5, compared to carriers of the wild-type alleles. This was not confounded by eczema, rhinoconjunctivitis or asthma by history or sIgE to the food tested by DBPCFC.

The association between having one or more *FLG* LOF variants and food allergy was significantly replicated using the family-based design, with a significant increase of transmission of one or more risk alleles to the DBPCFC-positive child (Z -score = 3.042, $P < 0.01$).

Data on sensitization were known for 148 children. Of these, 118 (79.7%) were sensitized to the tested food. Of these 118 sensitized children, 28 (23.7%) had at least one *FLG* LOF variant, compared to 4 (13.3%) of 30 nonsensitized children. There was no significant association between sensitization to foods and *FLG* LOF variants ($P = 0.22$, OR 2.02, 95% CI 0.65–6.29).

As the carriage of *FLG* LOF variants considerably increases the chance of being clinically reactive to foods, we examined whether including it in a model could increase the

Table 1 Descriptive statistics of the study population, classified according to phenotype ($n = 155$ M = missing)

	Clinically reactive to at least one food $N = 102$	Not clinically reactive to any food $N = 53$
R501X (% within genotype)		
C:C	90 (64.7)	49 (35.3)
T:C	10 (83.3)	2 (16.7)
M	2	2
R2447X (% within genotype)		
C:C	52 (34.7)	98 (65.3)
T:C	4 (100.0)	0 (0.0)
M	0	1
2282del4 (% within genotype)		
CAGT:CAGT	81 (63.3)	47 (36.7)
–:CAGT	14 (77.8)	4 (22.2)
–:–	3 (100.0)	0 (0.0)
M	4	2
S3247X (% within genotype)		
C:C	101 (65.6)	53 (34.4)
C:A	1 (100.0)	0 (0.0)
Summary genotype (% within genotype)		
0 Loss-of-function risk alleles	73 (59.8)	49 (40.2)
1+ Loss-of-function risk alleles	29 (87.9)	4 (12.1)
Age in months (SD)	93.6 (54.8)	81.6 (62.0)
Gender	Male: 54.9%	Male: 69.8%
	Female: 45.1%	Female: 30.2%
Suspected food	Peanut: 35.3%	Cow's milk: 43.4%
	Cow's milk: 28.4%	Peanut: 26.4%
	Hen's egg: 12.7%	Hen's egg: 15.1%
	Hazelnut: 9.8%	Cashew: 7.5%
	Cashew: 7.8%	Hazelnut: 3.8%
	Soy: 2.9%	Walnut: 1.9%
	Walnut: 2.9%	Soy: 1.9%
Specific IgE in kU/l ($\pm$)	25.05 (34.8)	3.64 (7.4)
	Specific IgE positive (>0.35)	31 (58.5%)
Specific IgE negative (<0.35)	9 (8.8%)	21 (39.6%)
Data on IgE levels missing	6 (5.9%)	1 (1.9%)
Eczema by history	Yes: 90(88.2%)	Yes: 41(77.4%)
	No: 8	No: 10
	Missing: 4	Missing: 2
Asthma by history	Yes: 62(60.8%)	Yes: 25(47.2%)
	No: 36	No: 26
	Missing: 4	Missing: 2
Rhinoconjunctivitis by history	Yes: 51(50.0%)	Yes: 15(28.3%)
	No: 45	No: 35
	Missing: 6	Missing: 3

M, missing.

positive predictive value of the resultant test to a level which could make DBPCFC testing unnecessary (Table 2). Adding *FLG* LOF variants improved the predictive model for clinical

Table 2 Predictive multivariate model for clinical reactivity using the *FLG* gene, eczema in history, specific IgE, and gender. The model can be used by the following equation: $-3.87 + 1.42 \times (1 \text{ for } FLG \text{ LOF variant, } 0 \text{ for wild type}) + 1.35 \times (1 \text{ for low sIgE, } 2 \text{ for moderate sIgE, } 3 \text{ for high sIgE, } 4 \text{ for very high sIgE}) + 1.58 \times (1 \text{ for eczema in history, } 0 \text{ for no eczema in history}) + 0.98 \times (1 \text{ for male, } 2 \text{ for female}) = X$. Predictive value for clinical reactivity = $E^X / (E^X + 1)$. Low sIgE is defined as values between <5 kU/l, moderate sIgE as 5–20 kU/l, high sIgE as 20–80 kU/l, and very high sIgE as >80 kU/l. The model is computed by binary logistic regression analysis assuring that the assumption of linearity in nondichotomous variables was met. The presence of one or more *FLG* LOF variants and sensitization or eczema by history are independent predictive variables as they all significantly predict food allergy in the same multivariable logistic regression model and their interaction term is not significant ($P = 0.91$ for *FLG* LOF and sensitization, $P = 0.99$ for *FLG* LOF and eczema). The model explained 35.5% (Nagelkerke R Square) of the variance in clinical reactivity. The area under the ROC curve was 0.81, with $P < 0.01$. Including the genotype makes the predictive model more accurate because the $-2 \log$ likelihood changed by 5.78 ($P = 0.02$) and the area under the ROC curve increased from 0.79 to 0.81. Specificity and positive predictive value were both positively related with the cutoff value, and sensitivity and negative predictive value were both negatively related with the cutoff value. Maximum specificity and positive predictive value were preferred above sensitivity and negative predictive value as the relative risk of *FLG* LOF variants is 1.5 and they are therefore likely to be important in 'ruling in' rather than 'ruling out' food allergy. Using the 0.90 cutoff value, 40 children in the study population were classified as being food allergic. This classification is correct in 37 cases (positive predictive value 92.5%, specificity 94.0%). Using the cutoff value of 0.96 in the predictive model, 18 children in the total study population were classified as being food allergic. This classification is correct in all cases, corresponding to a positive predictive value and specificity of both 100%. As a consequence, the negative predictive value and sensitivity were 40.0% and 20.0%, respectively

	P-value	OR	95% CI for OR Lower–upper
Presence of loss-of-function variants of the <i>FLG</i> gene	0.03	3.99	1.17–13.60
History: eczema	0.03	3.84	1.12–13.15
Specific IgE (low, moderate, high or very high)	0.00	3.92	2.04–5.90
Gender	0.04	2.48	1.04–5.90

OR, odds ratio; CI, confidence interval.

reactivity to foods. A high positive predictive value of this model was shown, with consequently a low negative predictive value.

Discussion

Of children suspected of being food allergic, those carrying at least one *FLG* LOF variant are 1.5 times more likely to be clinically reactive to foods as diagnosed by the DBPCFC compared to children carrying wild-type alleles. This strong association is replicated using a family-based design, confirming the robustness of this observation. No significant associa-

tion was identified between *FLG* LOF variants and sensitization.

In the genetic association studies on food allergy and *FLG* gene variants reported so far (3–5), food allergy was not exclusively ascertained by DBPCFC and/or included nonsensitized controls. Therefore, these studies may have failed to distinguish genetic associations with sensitization from associations with clinical reactivity as the false-positive rate in open food challenges is considerable, including those in young infants (9, 10). It has been reported that *FLG* LOF variants are associated with eczema and increased transepidermal water loss and may therefore favor the dual allergen-exposure hypothesis which leads to sensitization (1, 2, 5). A recent study showed that LOF *FLG* gene carriage was not associated with sensitization to foods, whereas the presence of eczema was (11). Here, we go on to show that *FLG* LOF variant carriage is significantly associated with clinical reactivity to foods. This study population was underpowered to replicate the association between *FLG* LOF variants and sensitization or eczema due to the small number of children without eczema and sensitization. Whether the association of *FLG* LOF with food allergy is truly independent from the presence of eczema and sIgE may only be unequivocally determined in a population with a lower prevalence of eczema and sensitization than that seen here.

This study is the first showing a possible role of a genetic marker, in this case the *FLG* gene, in the diagnosis of food allergy. As this model predicts clinical reactivity to a food, but not which food(s) are involved, its impact on clinical practice may be more limited than models predictive of allergy to specific foods. Nevertheless, these results demonstrate that genetic markers may contribute to models which aim to predict clinical reactivity in food allergy. Such a model may be useful as a diagnostic tool to reduce the need for DBPCFCs in children who are highly likely to be food allergic. Future studies will be needed to further refine the model for each specific food, to incorporate allergen-specific threshold values, and to replicate it in different populations.

In conclusion, we showed that loss-of-function variants of the *FLG* gene are strongly associated with clinical allergic reactivity to foods in our population of children highly suspected of being food allergic. Furthermore, genetic markers may be useful as an addition to clinical assessment in the diagnosis of food allergy.

Author contributions

Van Ginkel contributed to the data collection and analysis and wrote the manuscript. Flokstra-de Blok contributed to the data collection and manuscript. Kukler contributed to the data collection. Kollen contributed to the data analysis and manuscript. Koppelman supervised the study and contributed to data analysis and manuscript. Dubois supervised the study and contributed to manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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