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Associations between child filaggrin mutations and maternal diet with the development of allergic diseases in children

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Conflict of interest statement

Dr. Venter reports grants from Reckitt Benckiser, grants from Food Allergy Research and Education, grants from National Peanut Board, during the conduct of the study; provided educational maternal or consultancy for Reckitt Benckiser, Nestle Nutrition Institute, Danone, Abbott Nutrition, Else Nutrition, and Before Brands, outside the submitted work. Dr. O'Mahony reports personal fees from PrecisionBiotics, personal fees from Nestle, personal fees from Nutricia, personal fees from Reckitt, outside the submitted work. The other authors declare no interests.

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Abstract

Background

Filaggrin (FLG) loss-of-function mutations in children and maternal diet in pregnancy have been implicated in child allergy outcomes. This paper studies the questions: "Do FLG mutations modify the effect of maternal diet on the odds of development of allergic diseases?" and "Which factor leads to the highest rate of diagnosis allergic diseases over time, maternal diet, or FLG mutations?"

Methods

Exact logistic regressions studied effect modification. Cox proportional hazards models compared the rate of allergic disease development in three groups (N=624): (1) children with FLG mutation, (2) children without FLG mutation whose mothers did not eat an allergy preventive diet, and (3) children without FLG mutation whose mothers ate an allergy preventive diet. Maternal diet was classified using a validated index.

Results

There was no significant effect modification for any outcome. Cox models showed development of atopic dermatitis, asthma, and wheeze were significantly higher for children in group 1 vs. 3 (HR=2.40 [1.32, 4.37], HR=2.29 [1.05, 4.97], and HR 2.10 [1.004, 4.38], respectively), but not significantly higher for children in group 1 vs. 2 (HR=1.30 [0.74, 2.29], HR=1.27 [0.61, 2.63], and HR=1.29 [0.65, 2.58], respectively). Development of allergic rhinitis was significantly higher for group 1 vs. 2 and 3 (1 vs. 2: HR=2.29 [1.10, 4.76]; 1 vs. 3: HR=3.21 [1.46, 7.08]).

Conclusion

Child FLG mutation did not modify the effect of maternal diet. Children with FLG mutation had similar risk of atopic dermatitis, asthma, and wheeze as children without an FLG mutation whose mothers did not eat an allergy preventive diet during pregnancy. The results suggest that maternal diet in pregnancy, a modifiable risk factor, could be a target for preventive interventions.

Keywords: maternal diet, pregnancy, allergy, prevention, Filaggrin, atopic dermatitis, asthma, allergic rhinitis

Abbreviations

FLG: Filaggrin

FPQ: Food propensity questionnaire

DNA: Deoxyribonucleic acid

HR: Hazard ratio

Introduction

More than 50 million Americans suffer from allergies including asthma, wheeze, allergic rhinitis, atopic dermatitis and food allergy.[1] Although allergic disease is very common, the pathoetiology is not completely understood. Some factors are known to be deleterious. A disrupted epithelial barrier has been implicated in allergic diseases such as asthma, allergic rhinitis and eczema.[2] Both environmental factors and genetic risk factors may damage the epithelial barrier. The disrupted barrier may then allow exposure to environmental and dietary allergens, and the development of allergic disease.[2]

The filaggrin gene codes for structural proteins that are crucial for epidermal regulation and skin barrier function.[3] Loss-of-function mutations have been implicated in a variety of allergic diseases. Filaggrin (FLG) loss of mutations have been reported in 10 - 40% of children with atopic dermatitis.[4] A recent meta-analysis by Drislane et al.[4] showed that "across all studies there is a high risk independently conferred by both null polymorphisms, with an estimated overall OR of 3.51, 3.62, and 3.58 for R501X, 2282del4, and the combined genotype, respectively." FLG loss of function mutations have also been described as a risk factor for the development of asthma, allergic rhinitis and food allergy.[4,5]

Maternal diet in pregnancy has been implicated in the development of allergic outcomes.[6,7] Our team has previously demonstrated that a maternal diet rich in vegetables and yogurt and with reduced intake of red meat, cold cereal, fried potatoes, rice and grains, and 100% fruit juice was associated with 23% reduced odds of atopic dermatitis, 16% reduced odds of asthma, 18% reduced odds of allergic rhinitis, and 20% reduced odds of wheeze.[8] However, in this study,[8] the potential effects of FLG mutations was not considered.

Both FLG mutations in the child and maternal dietary intake in pregnancy have been implicated in child allergy outcomes. We set out to answer the questions: "Do FLG mutations modify the effect of maternal diet on child allergy outcomes?" and "Which confers more risk, child FLG mutations or maternal diet?"

Methods

This study leveraged previously collected data and biospecimens from an ongoing longitudinal epidemiological study (Healthy Start, Dabelea, Principal Investigator).[9,10] The Healthy Start study enrolled 1410 mother-child dyads. The analysis presented in this paper included 624 multi-ethnic mother-child dyads recruited during pregnancy with available data for maternal dietary intake

during pregnancy, DNA extracted from cord blood and analyzed for FLG mutation variants, and verified child allergic outcomes. The Healthy Start study protocol was approved by the Colorado Multiple Institutional Review Board. (IRB number: 09-0563; Healthy Start 1; 2009-2014 and Healthy Start 2; 2015-present). The Healthy Start study was registered as an observational study at clinicaltrials.gov as NCT02273297.

Maternal diet

A food propensity questionnaire (FPQ) was given to women to complete at both their mid-pregnancy and delivery research interviews. Each FPQ inquired about the women's diet over the past three months. Full details of the FPQ data appear in Venter et al.

A maternal diet index previously shown to be associated with prevention of allergy was computed. The maternal diet index included intake of vegetables, yogurt, fried potatoes, cold cereal, red meat, 100% pure fruit juice, and rice/grains. The maternal diet index was dichotomized at the median, and women with index scores greater than or equal to the median were classified as having allergy preventive diets. Women with index scores below the median were classified as having non-allergy preventive diets.[8]

Genotyping methods

We characterized FLG loss-of-function mutation variants in previously collected cord blood DNA for a subset of Healthy Start participants. Cord blood was collected for participants at delivery whenever possible. The child DNA was analyzed, in all samples with sufficient DNA for analysis for the following six FLG mutation variants: R501X, S3247X, R2447X, 2282del4, p.S3316X, and p.R826X. Genotyping was performed using Taqman allelic discrimination assays (Table E1 in Online Repository) on a Viia 7 instrument (Thermo Fisher). If a participant had a minor allele genotype for any of the six FLG mutations (Table 1), they were categorized as having at least one FLG mutation. Children with wild-type results for all six FLG variants were defined as having no FLG mutations.

Medical record verified child allergy outcomes

Participants provided consent for review of child medical records. The electronic medical records were searched using previously published terms used by a systematic review[6] for asthma, wheeze, allergic rhinitis, hay fever, seasonal allergies, atopic dermatitis and eczema. Detailed methods for the electronic medical record search can be found in Venter et al.[11], and are summarized below.

Child allergy outcomes included allergic rhinitis (including seasonal allergies and hay fever), atopic dermatitis (including eczema), asthma, and wheeze.[11] Medical records were reviewed by one of the researchers, who assigned diagnoses after thorough review of medical notes. Age at diagnosis was computed using the child's date of birth and the service date associated with their diagnosis. If the medical records of a participant did not match any of the search terms, the participant was considered to have no allergy diagnoses between birth and the age at which the medical record search was conducted. All children were counted in the analyses regardless of allergy status.

Demographic Data

Information regarding child race/ethnicity, nulliparity, maternal history of asthma, gestational smoking, breastfeeding duration, and age of introduction of solid foods were obtained via questionnaires. Mode of delivery and child's sex were obtained from electronic medical record data.

Statistical analysis

Demographic characteristics of participants included in the analytic sample were summarized using means and standard deviations or frequencies and percentages for continuous or categorical variables, respectively. Demographic characteristics were compared between those with no child FLG mutation and those with any FLG mutation using non-parametric Wilcoxon rank sum tests and Fisher's exact tests for continuous and categorical variables, respectively.

The duration of follow-up differed for each child as consent was either given to review the child's medical records from birth up to age 4 years, but not 4 to 8 years; or to review the child's medical records from birth up to age 8 years, but the child may not have yet reached 8 years of age when the records were searched. Since logistic models cannot account for different lengths of follow-up, the logistic models were fit only for data up to 4 years of age. The Cox models included data up to 8 years of age.

Separate exact logistic regression models were fit for each of the medical record verified childhood allergic disease outcomes (allergic rhinitis, atopic dermatitis, asthma, wheeze). The goal was to determine whether child FLG mutation modifies the effect of maternal diet on allergy outcomes. For each allergic disease, the outcome for the exact logistic regression models was diagnosis at any time up to age 4 years. The predictors for the models were maternal diet,

dichotomized at the median as allergy preventive or non-allergy preventive, presence of at least one FLG mutation, and the interaction between the diet and FLG terms. The interaction terms were assessed with exact categorical tests.

Cox proportional hazards models were used to examine the associations between any FLG mutation in the child, maternal diet, and allergic outcomes up to 8 years. The Cox proportional hazards modeling approach was used to censor participants up to the age for which they had available electronic medical record data. Age at their first diagnosis was used in all cases.
The rates of development of allergic rhinitis, atopic dermatitis, asthma, and wheeze were compared between the three child FLG mutation and maternal diet groups: (1) children with FLG mutation, (2) children without FLG mutation whose mothers did not eat an allergy preventive diet, and (3) children without FLG mutation whose mothers ate an allergy preventive diet. To understand the interaction between FLG mutation and diet, we would have had to use four groups, stratified by child FLG mutation status (yes vs. no), and diet (preventive vs. non-preventive). However, we were unable to divide the group of children with a FLG mutation into two groups based on maternal diet because the resulting sample sizes were too small to fit Cox proportional hazards models to test differences in rate of development of allergic diseases.

We conducted hypothesis tests and computed hazard ratios (HR) and 95% confidence intervals. For each allergic disease we fit unadjusted and adjusted models. The adjusted models included the following covariates: gestational smoking, nulliparity, maternal history of asthma, mode of delivery, breastfeeding duration, age of introduction of solid foods, child's race/ethnicity, and child's sex. The covariates were the same covariates as chosen in the development of the maternal diet index.[8] We checked that the assumption of proportional hazards was met prior to interpreting the results of these models. Significance for all statistical hypothesis testing was assessed at an alpha level of 0.05. No multiple comparisons adjustments were made, as all hypotheses were *a priori* questions of interest. As a sensitivity analysis, we adjusted the Cox proportional hazards models for season of birth, as a proxy for maternal prenatal vitamin D exposure.

As an exploratory analysis, we computed Fisher's exact tests to compare the cumulative incidence of each allergic disease between those with maternal diet index score ≥ median (allergy preventive) and maternal diet index score < median (non-allergy preventive), among children with a FLG mutation.

Results

The analytic sample included N=624 participants with available maternal diet data, child FLG genotype, and medical record allergy data (Figure 1). There were no significant differences in the demographic characteristics between the participants included in this analysis and those who were not due to missing data (data not shown). There were 49 children with a FLG mutation (8%). Table 2 describes the characteristics of the study participants, overall, and stratified by the presence or absence of a child FLG mutation.

There were no significant differences in maternal diet, breastfeeding duration, age of introduction of solid foods, gestational smoking, nulliparity, maternal history of asthma, mode of delivery, child's race/ethnicity, or child's sex between children with or without a FLG mutation. The frequency and percentage of children with each of the FLG mutation variants studied are presented in Table E2 (see Online Repository). The breakdown of child FLG mutation variants by race/ethnicity are shown in Table 3.

Results of the exact logistic regression models (Table 4) showed that the interaction between child FLG mutation status and maternal diet was not significant for any of the allergic disease outcomes.

Figure 2 displays the rates of development of (a) allergic rhinitis, (b) atopic dermatitis, (c) asthma, and (d) wheeze for the three groups. The plots in Figure 2 were from the adjusted Cox proportional hazards model fit for each of the outcomes, with covariates values set equal to their median value for continuous variables or their most frequent level for categorical variables. The plots for each of the allergic disease outcomes all show a similar pattern of results. For all allergic outcomes, the highest rate of disease occurs in group 1, children with a FLG mutation. The middle rate of disease occurs in group 2, children without FLG mutation whose mothers did not eat an allergy preventive diet. The lowest rate of disease occurs in group 3, children without FLG mutation whose mothers ate an allergy preventive diet.

Table 5 presents the results of the unadjusted and adjusted Cox proportional hazards models examining the difference in the rate of disease development between the genetic and dietary groups. For all allergic disease outcomes, children in group 1 (FLG mutation) had a significantly increased rate of disease development compared to children in group 3 (no mutation, preventive

diet) (HR [95% CI]: allergic rhinitis = 3.21 [1.46, 7.08]; atopic dermatitis = 2.40 [1.32, 4.37]; asthma = 2.29 [1.05, 4.97]; wheeze = 2.10 [1.004, 4.38]).

For atopic dermatitis, asthma, and wheeze, there was no significant difference between children in group 1 (FLG mutation) compared to children in group 2 (no mutation, non-preventive diet) (HR [95% CI]: atopic dermatitis = 1.30 [0.74, 2.29]; asthma = 1.27 [0.61, 2.63]; wheeze = 1.29 [0.65, 2.58]. For the outcome of allergic rhinitis, children in group 1 (FLG mutation) had a significantly higher rate of disease development compared to children in group 2 (no mutation, non-preventive diet) (HR [95% CI] = 2.29 [1.10, 4.76]).

For atopic dermatitis and asthma, children in group 2 (no mutation, non-preventive diet) had a significantly greater rate of disease development compared to children in group 3 (no mutation, preventive diet) (HR [95% CI]: atopic dermatitis = 1.85 [1.23, 2.78]; asthma = 1.80 [1.07, 3.02]). The sensitivity analysis showed that additional adjustment for the season of birth of the offspring, a proxy for maternal prenatal vitamin D exposure, did not change the direction, magnitude, or significance of our results (data not shown).

The exploratory analysis showed that among children with a FLG mutation, the cumulative incidence of asthma was significantly higher for those with a non-allergy preventive maternal diet in pregnancy compared to those with an allergy preventive maternal diet (44% vs. 10%, p = 0.01). Although the cumulative incidences of allergic rhinitis, atopic dermatitis, and wheeze were also observed to be greater among children with a FLG mutation than for those with a non-allergy preventive maternal diet than those with an allergy preventive maternal diet, the differences were not statistically significant (see Table E3 in Online Repository).

Discussion

In this study we compared the magnitude of the association between child FLG mutations and incidence and timing of offspring allergic diseases and a maternal allergy preventive diet during pregnancy and incidence and timing of offspring allergic diseases during childhood. Carrying a FLG mutation has been directly associated with significantly increased risk of childhood eczema due to its effect on skin barrier function, and indirectly with other allergic diseases following eczema as part of the allergic march. Rates of outcomes were lowest in children without FLG mutation whose mothers ate an allergy preventive diet during pregnancy. Children with FLG mutation had similar risk of atopic dermatitis, asthma, and wheeze as children without an FLG

mutation whose mothers did not eat an allergy preventive diet during pregnancy. This result suggests that in children with no FLG mutation, the risk conferred by not eating an allergy preventive diet during pregnancy was similar to the risk conferred by having a FLG mutation.

In our cohort, 8% of children showed a filaggrin gene mutation. Two UK birth cohort with predominantly Caucasian participants reported that 11.8%[12] and 10.3%[13] of their populations had a filaggrin gene mutation using the same genes and analysis as our cohort. The slightly lower percentage seen in this study probably reflects the strong population admixture seen in the Denver metropolitan area, with children of ethnic ancestries from across the globe. There is the possibility that some individuals with rare FLG mutations that are not captured by the allelic discrimination assays used in this study[14] will be misclassified as wildtype, this is unlikely to alter the conclusions of the study as the FLG variants assessed in this capture the most common variants in both European and African-American populations.

Very little data is available about the mechanisms underlying the association between dietary intake in pregnancy and child allergic outcomes. One study suggested that glutamine supplementation may affect allergy outcomes and reduced inflammatory T-cell responses in cases with Caspase Recruitment Domain Family Member 11 (CARD11) gene mutations.[15] Another study indicates that omega-3 fatty acid intake in pregnancy was associated with reduced child eczema through epigenetic changes associated with the fatty acid desaturase 1 and 2 (FADS1 and 2) and Fatty Acid Elongase 5 (ELOVL5) genes.[16] Other hypotheses include the effect of specific micronutrients on the development of the immune system and the effect of maternal diet on maternal microbiota.[17,18]

One question often encountered in a clinical allergy setting is if a genetic risk of developing an allergy, outweighs the risk of a healthy diet. The data from this study indicates that genetic risk may outweigh the risk of a non-allergy prevention diet for child allergic rhinitis, but both genetic risk and non-allergy prevention diets confer similar risk for child asthma, wheeze and eczema. To the best of our knowledge, this is a novel finding and there are no previous studies focusing on child risk of allergy outcomes, child genetic risk and maternal overall dietary intake in pregnancy.

Two studies focusing on environmental exposure to peanut demonstrated that peanut levels in dust in early life are associated with an increase in the development of peanut allergy, especially if the infant had filaggrin loss-of-function mutations or a history of eczema,[19,20] but did not assess

the association between FLG mutations, maternal food intake during pregnancy and allergy outcomes. These authors hypothesize that the effect seen may indicate that children with a skin barrier dysfunction, may be more like to become sensitized to food allergens via a defective skin barrier function. An alternative hypothesis is that household dust levels might be another marker of the contact from household peanut consumption on the environment and skin of the child.[21]

Both genetic and dietary effects are important in precision nutrition. Genetic and dietary effects have been studied for many of the non-communicable diseases such as obesity, diabetes, cardiovascular diseases, cancer and respiratory diseases.[22] However, relatively little is known the comparative effects of genetic and dietary factors in allergy prevention.[16] We studied dietary exposures during pregnancy and one of the most common genetic mutations associated with allergy outcomes, due to its importance in epithelial barrier function.[23] We classified children by FLG mutation status and maternal dietary intake during pregnancy to provide information on how genotypic information could be used to inform nutritional recommendations in pregnancy for allergy prevention. Using genotypic information obviates biases which can be created by using self-reported variables, such as maternal history of allergy. Other strengths of the study include the large cohort that has been followed prospectively and allergy diagnosis based on electronic medical record review. There is currently no data to address the sensitivity and specificity of this approach, however medical record data have been used in European cohort studies to report on asthma outcomes and to validate these methods of reporting.[24]

Results of the sensitivity analysis showed that additionally adjusting for child's season of birth, as a proxy for maternal prenatal vitamin D exposure, did not change the results. This indicates little support for the hypothesis that maternal vitamin D levels affect the risk of offspring disease outcomes. Furthermore, we did not adjust our data for childhood vitamin D intake as current information on vitamin D intake in infancy and childhood and its association with allergy outcomes provides a confusing picture. [25-32] This may be due to various factors that can influence vitamin D levels such as sun exposure, country of residence, ethnicity, age, diet intake, vitamin D supplementation (timing, formulation and dose), genetic polymorphisms affecting vitamin D metabolism, epigenetic changes that contribute to vitamin D levels, vitamin D binding protein, interaction with disease-associated genetic polymorphisms, definition of vitamin D insufficiency/deficiency, and time-points for assessment of vitamin D status.[33] We acknowledge that are data suggesting that vitamin D intake in childhood may increase the risk of asthma. A prospective birth cohort study of 123 infants indicated that vitamin D intake of >13

ug/day were associated with increased asthma by 6 years of age.[34] Another study[35] reported that children supplemented with vitamins A and D in water-soluble form, as opposed to a fat soluble form, during infancy showed an increased risk of asthma by 4 years of age. We did not have this level of detailed information on vitamin D intake in children.

There were some limitations of the study. Because there was only a small subset of children with a FLG mutation (n=49), we were unable to examine the interaction between dichotomous maternal diet index and child FLG mutation status in the Cox proportional hazards models as there were cell sizes less than five. However, we were able to perform exact logistic regression analyses to test this interaction and the results indicated that there was no significant interaction between dichotomous maternal diet index and child FLG mutation status for child allergic diseases up to age 4 years. In order to answer this question in a true precision nutrition fashion, a study recruited on the basis of FLG mutation is necessary to provide the sample size to study the interaction in detail. Further limitations include low prevalence of food allergy in this cohort and a lack of skin prick test to confirm atopic status. We were unable to evaluate IgE-mediated food allergy as an outcome for the Cox proportional hazards models because the small number of children diagnosed with IgE-mediated food allergy that also had a FLG mutation (n=2) prevented these models from being fit. In addition, we were unable to examine IgE-mediated food allergy as an outcome in the exact logistic regression models that tested the interaction between maternal diet and child FLG mutation, because there were no children with IgE-mediated food allergy who had an FLG mutation and a maternal diet index score below the median. While the study did not account for childhood diet, the analysis did adjust for factors previously shown to be important in allergy risk,[8] including gestational smoking, nulliparity, mode of delivery, breastfeeding duration, age of introduction of solid foods, child's race/ethnicity, and child's sex.

For allergy prevalence data, children were followed-up at standard pediatric visits in the US, and for any other medical condition that required a medical assessment at Children's' Hospital Colorado, including allergy assessments. We are aware of the limitations of using electronic medal record data opposed to objectively diagnosed data. There is currently no data to address the sensitivity and specificity of this approach, however medical record data have been used in European cohort studies to report on asthma outcomes and to validate these methods of reporting.[24] There are limited studies or surveys from the US using medical record data such as the data present by Hill et al.[36] but this approach was not validated.

Data in terms of “ever” allergy, transient allergy and persistent allergy was not available for our cohort. In terms of atopic dermatitis, there seems to be inconsistency of findings about the association between FLG-LOF and different atopic dermatitis phenotypes with one study showing an inconsistent association between FLG genotype, an different definitions of atopic dermatitis.[37] Data from a UK and European cohort showed an association between FLG-LOF mutations and all definitions of atopic dermatitis (no atopic dermatitis, Early-onset-persistent atopic dermatitis, Early-onset-late-resolving atopic dermatitis, Early-onset-early-resolving atopic dermatitis, Mid-onset-resolving atopic dermatitis, Late-onset-resolving atopic dermatitis) other than mid childhood onset of atopic dermatitis (UK cohort) and children with early onset atopic dermatitis who outgrew their atopic dermatitis (EU cohort).[38] In terms of asthma, data from the COPSAC study (Denmark)[39] indicated an increased risk of developing recurrent wheeze, asthma and asthma exacerbations in children with FLG-LOF mutations in the 1.5 yr of life. FLG-LOF increased the risk of eczema in the first year of life. The COPSAC study therefore shows that FLG-LOF is not only associated with the development of allergic diseases but also the timing of development, recurrence of disease and severity.

Infant diet may also affect allergy outcomes, but we were unable to take infant diet into account in our analysis other than breastfeeding duration and age of introduction of solids. Further gaps that need to be addressed in future include addressing questions about the association between dietary intake during pregnancy, its association with the maternal and infant microbiome, maternal and child epigenetic profiling and subsequent child allergic outcomes.

In conclusion, the results of the study imply that not eating an allergy preventive diet in pregnancy confers similar risk as having the FLG loss of function mutation for the development of child asthma, wheeze and eczema. The lowest risk occurred in children without FLG mutation whose mothers ate an allergy preventive diet during pregnancy. Maternal diet during pregnancy is a modifiable risk factor which can be addressed to reduce offspring allergic disease risk.

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Impact statement

Our results show that child FLG mutations do not modify the strength of the association between maternal diet and child allergy outcomes. In addition, we show that FLG mutations and a maternal non-allergy preventive diet during pregnancy confer similar risk for offspring asthma, wheeze and atopic dermatitis. The results suggest that mothers, regardless of FLG mutation status, should be counseled regarding dietary intake in pregnancy.

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Tables

Table 1. Classification of genotypes as wild-type or mutant for each of the Filaggrin mutations analyzed

Mutation name	dbSNP ID	Wild-type genotype	Mutant (minor allele) genotype(s)
R501X	rs61816761	Homozygous allele 1/allele 1	Homozygous allele 2/allele 2, Heterozygous
S3247X	rs150597413	Homozygous allele 2/allele 2	Heterozygous
R2447X	rs138726443	Homozygous allele 1/allele 1	Heterozygous
2282del4		Homozygous allele 1/allele 1	Heterozygous
p.S3316X	rs149484917	Homozygous allele 1/allele 1	Heterozygous
p.R826X	rs115746363	Homozygous allele 1/allele 1	Heterozygous

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Table 2. Descriptive characteristics of study participants

	Overall sample	No child Filaggrin mutation	Yes child Filaggrin mutation†	
Sample size (N)	624	575	49	
Continuous variables	← median (interquartile range) →			p-value‡
Breastmilk-months	7.00 (2.00, 12.75)	7.00 (2.00, 12.63)	5.38 (1.52, 13.00)	0.86
Age of introduction of solid foods (months)	6.00 (5.00, 6.00)	6.00 (5.00, 6.00)	6.00 (4.00, 6.00)	0.73
Categorical variables		← n (%) →		p-value§
Maternal diet index ≥ median	312 (50)	281 (50)	31 (63)	0.07
Gestational smoking	56 (9)	50 (9)	6 (12)	0.43
Nulliparous	314 (50)	286 (50)	28 (57)	0.37
Maternal history of asthma	101 (16)	91 (16)	10 (20)	0.42
Mode of delivery – vaginal	491 (79)	453 (79)	38 (78)	0.86
Child's race/ethnicity				0.67
Non-Hispanic White	323 (52)	293 (51)	30 (61)	
Non-Hispanic Black	77 (12)	72 (13)	5 (10)	
Hispanic	147 (24)	138 (24)	9 (18)	
Other¶	77 (12)	72 (13)	5 (10)	
Child's sex – female	295 (47)	270 (47)	25 (51)	0.66

† Children were classified as having a Filaggrin mutation if they had a non-wild type genotype for any of the following Filaggrin mutation variants: R501X, S3247X, R2447X, 2282del4, p.S3316X, p.R826X

‡ Group differences were assessed using non-parametric Wilcoxon rank sum tests.

§ Group differences were assessed using Fisher's exact tests.

¶ Other race/ethnicity includes Asian, American Indian/Alaska Native, Hawaiian/Pacific Islander, and multiracial.

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Table 3. Number of children with mutant genotypes for each of the Filaggrin mutation variants studied, stratified by child race/ethnicity

	N	R501X	S3247X	R2447X	2282del4	p.S3316X	p.R826X
Non-Hispanic white	323	14	2	2	9	0	4
Non-Hispanic black	77	1	1	0	2	0	1
Hispanic	147	0	1	0	5	0	4
Other†	77	1	1	0	2	1	0
Total with mutant genotype	624	16	5	2	18	1	9

† Other race/ethnicity includes Asian, American Indian/Alaska Native, Hawaiian/Pacific Islander, and multiracial.

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Table 4. Exact logistic regression models for diagnosis of allergic disease outcomes up to age 4 years that examine the interaction between child Filaggrin mutation status and maternal diet

Allergic disease	Exact odds ratio for interaction†	Exact 95% CI‡	Exact p-value
Allergic rhinitis	0.90	0.13, 5.65	1.00
Atopic dermatitis	0.98	0.21, 4.31	1.00
Asthma	0.55	0.07, 3.37	0.72
Wheeze	1.00	0.18, 5.29	1.00

† Odds ratio estimate is for the parameter for the multiplicative interaction between child Filaggrin mutation status (yes vs. no) and maternal diet (allergy preventive vs. non-allergy preventive)

‡ CI: confidence interval

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Table 5. Results of hypothesis tests in unadjusted and adjusted CPH models comparing rate of offspring development of allergic diseases between the three child Filaggrin (FLG) mutation and maternal diet index groups

	Unadjusted (N=624)			Adjusted† (N=492)		
	Groups‡ compared			Groups‡ compared		
	Group 1[c] vs. Group 2[d] (ref§)	Group 1 vs. Group 3[e] (ref§)	Group 2 vs. Group 3 (ref§)	Group 1 vs. Group 2 (ref§)	Group 1 vs. Group 3 (ref§)	Group 2 vs. Group 3 (ref§)
Allergic disease	← Hazard Ratio (95% CI¶) →			← Hazard Ratio (95% CI¶) →		
Allergic rhinitis	1.36 (0.71, 2.62)	3.20 (1.55, 6.61)**	2.35 (1.42, 3.87)***	2.29 (1.10, 4.76)*	3.21 (1.46, 7.08)**	1.40 (0.76, 2.57)
Atopic dermatitis	0.95 (0.57, 1.56)	2.45 (1.42, 4.21)**	2.59 (1.84, 3.64)***	1.30 (0.74, 2.29)	2.40 (1.32, 4.37)**	1.85 (1.23, 2.78)**
Asthma	0.99 (0.53, 1.88)	2.36 (1.18, 4.71)*	2.37 (1.54, 3.65)***	1.27 (0.61, 2.63)	2.29 (1.05, 4.97)*	1.80 (1.07, 3.02)*
Wheeze	0.96 (0.52, 1.77)	2.19 (1.14, 4.24)*	2.28 (1.52, 3.41)***	1.29 (0.65, 2.58)	2.10 (1.004, 4.38)*	1.62 (0.995, 2.64)

† Adjusted for the following covariates: gestational smoking, nulliparity, maternal history of asthma, mode of delivery, breastfeeding duration, age of introduction of solid foods, child's race/ethnicity, and child's sex

‡ Group 1: children with FLG mutation; Group 2: children without FLG mutation whose mothers did not eat an allergy preventive diet; Group 3: children without FLG mutation whose mothers ate an allergy preventive diet

§ The reference group is indicated with the abbreviation "ref"

¶ CI: confidence interval

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

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Figure legends

Figure 1. Participant exclusion diagram for the analytic sample.

Figure 2. Comparing rates of (a) allergic rhinitis, (b) atopic dermatitis, (c) asthma, and (d) wheeze development in offspring between the three child Filaggrin mutation and maternal diet groups. Plots produced from models adjusted for covariates with their values set equal to their median value for continuous variables or their most frequent level for categorical variables.