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Gene Polymorphisms, Breastfeeding and Development of Food Sensitization in Early Childhood

Xiumei Hong, MD, PhD^{1,†}, Guoying Wang, MD, PhD¹, Xin Liu, MD, PhD¹, Rajesh Kumar, MD, MS⁴, Hui-Ju Tsai, PhD^{1,3}, Lester Arguelles, PhD¹, Ke Hao⁵, Colleen Pearson, BA², Kathryn Ortiz, BA², Anthony Bonzagni, BA², Stephanie Apollon, BA², Lingling Fu, MS², Deanna Caruso¹, Jacqueline A Pongracic, MD⁴, Robert Schleimer, PhD⁶, Patrick G. Holt, DSc⁷, Howard Bauchner, MD², and Xiaobin Wang, MD, ScD¹

¹Mary Ann and J. Milburn Smith Child Health Research Program, Department of Pediatrics, Northwestern University Feinberg School of Medicine and Children's Memorial Hospital and Children's Memorial Research Center, Chicago, IL, USA

²Department of Pediatrics, Boston University School of Medicine and Boston Medical Center, Boston, MA, USA

³Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan

⁴Division of Allergy and Immunology, Children's Memorial Hospital, Chicago, IL, USA

⁵Merck Research Labs, 33 Ave. Louis Pasteur, Boston, MA 02115

⁶Division of Allergy-Immunology, Northwestern University Feinberg School of Medicine, Chicago, IL

⁷Telethon Institute for Child Health Research, and Centre for Child Health Research, University of Western Australia, Perth, Australia

Abstract

Background—The impact of breastfeeding on the development of allergic disease is uncertain. There are no data that show whether this relationship varies by individual genotypes.

Objective—To evaluate the effect of breastfeeding and gene-breastfeeding interactions on food sensitization (FS) in a prospective U.S. birth cohort.

Methods—This study included 970 children who were prospectively followed since birth. Breastfeeding history was obtained from a standardized questionnaire interview. FS was defined as specific IgE ≥ 0.35 kU_A/L to any of eight common food allergens. Eighty-eight potentially functional SNPs were genotyped from 18 genes involved in innate immunity or T_H1/T_H2 balance. Logistic regression models were used to test the effects of breastfeeding and gene-breastfeeding interactions on FS, with adjustment for pertinent covariates.

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[†]Correspondence and reprint requests should be addressed to: Xiumei Hong, MD, PhD, Mary Ann and J. Milburn Smith Child Health Research Program, Children's Memorial Hospital and Children's Memorial Research Center, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA, Telephone: 312-573-7735, Fax: 312-573-7825, xhong@childrensmemorial.org.

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Results—Children who were ever breastfed (n=739), including exclusively breastfed children, were at a 1.5 (95%CI=1.1-2.1, p=0.019) times higher risk of FS than never breastfed children (n=231). This association was significantly modified by rs425648 in the *IL12RB1* gene ($p_{\text{interaction}}=0.0007$): breastfeeding increased the risk of FS (OR=2.0, 95%CI=1.4-3.1, p= 0.0005) in children carrying the GG genotype but decreased the risk (OR=0.6, 95%CI=0.3-1.4, p=0.252) in children carrying the GT/TT genotype. Similar interactions were observed for SNPs in the *TLR9* (rs352140) and *TSLP* (rs3806933) genes. The interaction between the combined genotypes of the three SNPs and breastfeeding on FS was even stronger ($p_{\text{interaction}} < 10^{-5}$).

Conclusion—Our data suggest that the effect of breastfeeding on FS was modified by SNPs in the *IL12RB1*, *TLR9*, and *TSLP* genes both individually and jointly. Our findings underscore the importance of considering individual genetic variations in assessing this relationship.

Keywords

Breastfeeding; food sensitization; gene-environment interaction

Introduction

Food allergy, which affects both children and adults and may be increasing in prevalence, is emerging as a major clinical and public health problem worldwide^{1, 2}. In the latest *Guidelines for the Diagnosis and Management of Food Allergy in the United States*, it is recommended that all infants, including those with a family history of atopic disease, be exclusively breastfed until 4 to 6 months of age, unless breastfeeding is contraindicated for medical reasons³. However, the protective role of breastfeeding in preventing allergic diseases is uncertain. A large number of such studies have been conducted during the past decades, and the findings from these studies have been inconclusive with protective⁴⁻⁶, neutral⁷⁻⁹ and allergy-promoting effects¹⁰⁻¹² reported. Recently, a large prospective study demonstrated that early exposure to cow's milk (i.e., within 14 days of life) may have a protective effect on the development of milk allergy¹³.

Delineating the relationship between breastfeeding and allergic disease is clinically important, but challenging. Several methodological issues have been noted and need to be addressed in future studies. Reverse causation, which is mainly based on the premise that mothers might prolong breastfeeding if there are early signs of allergy in their children, is a major concern^{14, 15}. Misclassification of infant feeding history, due to recall bias and/or different measurements of breastfeeding across studies, is another issue in observational studies. Furthermore, recent studies reported that the association between breastfeeding and allergic diseases may vary by the type of allergic disease¹⁶, and by other factors including children's age¹⁷, gender¹⁸, and family history of allergy^{18, 19}, which may limit generalization of the findings from one specific allergic disease to another, and/or from one population to another.

There is growing evidence that gene-environment interactions are among the critical determinants of allergic susceptibility²⁰. For example, the interactions between microbial load and *CD14* genetic variants on allergic diseases have been reported and replicated in different studies²⁰. Gene-breastfeeding interactions have been found to affect growth rate²¹ and intelligence²². However, no such studies have been performed to look at allergic diseases or their related phenotypes. Further exploration in this area may provide new insights into the inconsistent findings on breastfeeding and allergic diseases as reported in the literature, and help to develop evidence-based clinical and public health recommendations.

Food sensitization (FS) in early childhood, defined by *in vitro* measurements of food-specific IgE (i.e. specific IgE >0.35 kU_A/L to a food allergen), is an important precursor of food allergy and other allergic diseases^{23, 24}. Although IgE titers fluctuate with age during early childhood²⁵, FS defined at age 2 years or beyond is much more likely to represent stable/persistent FS. As reported in a recent large German birth cohort, early-onset FS defined at age 2 years was an independent predictor of doctor-diagnosed food allergy at the age of 6 years²⁴.

The purpose of this study was to investigate the effect of breastfeeding on the development of FS in a prospective U.S. birth cohort. In particular, we explored whether this relationship was modified by an array of functional single nucleotide polymorphisms (SNPs) in 18 genes, which are known to play critical roles in innate immunity (i.e. *CD14*, *TLR2*, and *TLR4*), or in T_H1/T_H2 balance (i.e. t-box 21 [*TBX21*], interleukin receptor 12β [*IL12RB*], and thymic stromal lymphopoietin [*TSLP*]).

Materials and Methods

Study population

This study included 970 children from the Boston Birth Cohort, a cohort consisting of multi-ethnic mother-infant pairs (predominantly African American) enrolled 24 to 72 hours post-delivery and followed prospectively from birth onward as detailed in a previous publication²⁶. The information collected at birth includes comprehensive pre- and peri-natal epidemiological and clinical variables. Since 2004, infants who obtain primary or specialist care at Boston University Medical Center (BUMC) have been invited to participate in the post-natal Children's Health Study, a follow-up study to determine post-natal growth, development and health outcomes. The follow-up visits are scheduled at 6-12 months, 2, 4 and 6 years, consistent with the pediatric primary care visit schedule. Venous blood samples are collected at follow-up visits. The study protocols were approved by the Institutional Review Boards of Children's Memorial Hospital (CMH) in Chicago and BUMC.

Definition of breastfeeding history

Information on breastfeeding was obtained by a standardized questionnaire interview performed at study visits in the first few years of life. Specifically, mothers of the enrolled children were asked the question: "Did you breastfeed or formula feed your child?". The mother could answer (1) formula exclusively; (2) breastfed exclusively; or (3) both. Ever breastfed was defined if the mother reported "breastfed exclusively" or "both". The mothers who had ever breastfed their children at any point were further asked, "How long did you exclusively breastfeed for (no formula)?" and, "When was solid food first introduced to the baby?" If any solid food was introduced to the baby prior to the stated time of exclusive breastfeeding cessation, then exclusive breastfeeding duration was set as missing (n=12). For those subjects with multiple follow-up visits in the first few years of life, responses to the first survey were used.

Early signs of allergy

Maternal history of allergy was defined as mothers' self-reported history of allergic disease (including asthma, eczema, allergic rhinitis and food allergy). Family history of allergy was defined as children with at least one family member (including parents and older siblings) who had a history of allergic disease as defined above. Allergic disease in the first four months of life was defined as having a physician-diagnosed allergic disease (including eczema and food allergy, the two allergic diseases most likely to occur in the first year of life) in the first four months of life. This information was obtained by reviewing the medical record of each child.

Measurement of total and specific IgE

Cord blood total IgE (CBIgE) concentration in plasma was measured by the Clinical Immunology Laboratory at CMH using Phadia ImmunoCAP Total Low Range Assay (Phadia AB, Uppsala, Sweden), as reported previously²⁷. In early childhood, specific IgE (sIgE) concentration in plasma for each of 8 food allergens (egg white, cow's milk, peanut, soy, shrimp, walnut, wheat and cod) was measured using ImmunoCAP® at Quest Diagnostics according to the manufacturer's prescribed protocol. The detection limit was 0.35-100 kU_A/L. FS was defined as sIgE $\geq$ 0.35 kU_A/L to any of the 8 food allergens.

Candidate genes and functional single nucleotide polymorphisms (SNPs)

A total of 18 candidate genes, which encode the molecules that are critical to innate immunity and T_H1/T_H2 balance based on the literature, were analyzed in this study. For each gene, we selected potentially functional SNPs with a minor allele frequency (MAF) >0.05 , that met the following criteria: 1) coding SNPs; 2) SNPs creating/disrupting a splicing site; 3) SNPs predicted to be functional variants based on bioinformatics tools (such as PupaSuite (<http://pupasuite.bioinfo.cipf.es/>), FuncPred (<http://snpinfo.niehs.nih.gov/snpfunc.htm>) and F-SNP (<http://compbio.cs.queensu.ca/F-SNP/>)). For example, this included SNPs in transcription factor binding sites (TFBS), in exonic splicing enhancers (ESE) or silencers (ESS), in microRNAs and their target sequences (miRNA), and/or in a DNA triplex (triplex); and 4) SNPs within expression quantitative trait locus (eQTL). Ultimately, 98 SNPs with high Illumina design score ability (i.e., designability rank=1 and SNP score ≥ 0.60) were genotyped for this study.

Genotyping

SNPs were genotyped in the genotyping center at Washington University at St. Louis, using the Illumina GoldenGate custom panel. For quality control and quality assurance, four duplicate DNA control samples were included in each 96-well plate and were genotyped. The concordance rate of these duplicate samples was $>99.5\%$. Eighty-eight SNPs (89.8%) had a call rate $>98.0\%$ and thus were analyzed in the present study.

Ancestral proportion

A total of 150 ancestry informative markers (AIMs), with averaged δ (allele frequency difference between two ancestral populations) ≥ 0.5 , were randomly selected from a recently reported genome-wide admixture map²⁸. One hundred and forty-four AIMs had a call rate $>98.0\%$ in the total samples. Using the STRUCTURE program (version 2.3.1, <http://pritch.bsd.uchicago.edu/structure.html>), individual ancestral proportion for three ancestral populations (Asian, European and African) was calculated based on these 144 AIMs, and was then included as a covariate in subsequent analyses.

Statistical Analysis

We analyzed breastfeeding in two ways: ever breastfed (ever/never) and exclusive breastfeeding duration (≥ 4 months/ <4 months/never breastfed). Logistic regression models were applied to explore the associations between the two breastfeeding measures and FS (the primary binary outcome), with adjustment for maternal age at delivery, family history of allergy, maternal education, maternal smoking during pregnancy and/or after delivery, children's age (when FS status was defined), gender, ancestral proportion, allergic diseases in the first four months of life, and pets in the first year. To explore the possibility of reverse causation, we examined whether the two breastfeeding measures were associated with each of the early signs of allergy (maternal/family history of allergy, detectable vs. non-detectable CBIgE, and allergic disease in the first 4 months of life). Next, we conducted stratified

analyses to examine whether the associations between the two breastfeeding measures and FS varied by any of the early signs of allergy.

For each SNP genotyped in this study, a chi-squared test was applied to test Hardy-Weinberg equilibrium (HWE) in the total population (and in African Americans), and those that deviated from HWE ($p < 0.001$) were removed from this analysis. Pairwise linkage disequilibrium (LD) of SNPs in each gene was calculated using the PLINK program (<http://pngu.mgh.harvard.edu/~purcell/plink/>). With adjustment for the above-mentioned covariates, the interaction between each SNP and the breastfeeding measures was tested by including a product term in the logistic regression model and the p-value of the Wald test for this interaction term was reported. For each SNP, the best-fitting genetic model (additive, dominant or recessive), which was determined based on the Akaike information criterion, was applied. The false discovery rate (FDR) method was applied to correct for multiple testing²⁹. All data analyses were conducted using SAS software version 9.2 (SAS Institute, Cary, North Carolina, USA) and in R project (<http://www.r-project.org/>, version 2.8.1).

Results

A total of 970 children, who have been followed for an average of 2.5 ± 2.2 years, were included in this study. Three hundred and sixty-one children (37.2%) had FS. Seven hundred and thirty-nine children (76.2%) were ever breastfed, and 200 children were exclusively breastfed for ≥ 4 months. Ever breastfed children were more likely to have a non-smoking mother who was Hispanic, older at delivery, and had a higher education level than never breastfed children ($p < 0.05$) (Table 1). The additional variables, including children's gender and age when FS was defined, maternal/family history of allergy, allergic disease in the first four months of life and detectable vs. non-detectable CBIgE, showed no difference between these two groups.

Relationship between Breastfeeding and FS

As shown in Table 2, the prevalence of FS in breastfed children (39.6%) was higher than that for never breastfed children (29.4%, $p = 0.005$). With adjustment for the pertinent covariates, we found that ever breastfed children were at a 1.5 times (95%CI=1.1-2.1, $p = 0.019$) higher risk of FS than never breastfed children. The effect of exclusive breastfeeding for ≥ 4 months on the risk of FS (OR=1.6, 95%CI=1.1-2.5, $p = 0.029$) is comparable with that of exclusive breastfeeding for < 4 months (OR=1.5, 95%CI=1.0-2.2, $p = 0.034$).

Since FS status defined at age ≥ 2 years may be more stable and persistent, we then stratified the total sample into two groups according to the age when FS status was defined. We observed that the positive association between breastfeeding and FS was stronger in children aged ≥ 2 years (OR=2.3, 95%CI=1.3-4.1) than in those aged < 2 years (OR=1.1, 95%CI=0.7-1.8). There was no age-breastfeeding interaction ($p = 0.13$). Similar results were found for exclusive breastfeeding duration (Table 2).

Any Effect Modification by Early Signs of Allergy?

Early signs of allergy, including family (or maternal) history of allergy, detectable vs. non-detectable CBIgE and allergic disease in the first four months of life were evaluated in this study. In this cohort, 448 children had a family history of allergy. The percentage of ever breastfeeding was 73.7% and 78.4%, respectively, in children with and without a family history of allergy, which showed no difference ($p = 0.23$). The exclusive breastfeeding duration also was comparable in ever breastfed children with and without a family history of

allergy (Mean $\pm$ standard deviation [SD] = 3.3 ± 4.0 months and 2.9 ± 3.7 months, respectively, $p=0.16$ for t-test). The relationship between the two breastfeeding measures and FS did not vary by family history of allergy, which indicated no effect modification (Figure 2). Similar results were found for the other two early signs of allergy (Figure 2).

Gene-breastfeeding Interactions on FS

All 88 SNPs, which were successfully genotyped in this study with a call rate >0.98 , were under HWE. Thirteen SNPs, either with $MAF < 0.05$ ($n=5$) or in high LD with other SNPs ($r^2 > 0.8$, $n=8$), were excluded from further analyses (Table E1 in the Online Repository).

Table 3 lists the statistically significant gene-breastfeeding interactions on FS using a nominal significance level of 0.05. SNP rs425648 in the *IL12RB1* gene remained statistically significant after FDR correction (p_{FDR} for interaction = 0.04): ever breastfeeding was significantly associated with a 2.0 (95% CI = 1.4–3.1, $p=0.0005$) times increased risk of FS in children carrying the *IL12RB1*-rs425648 GG genotype, while this association was reversed in those carrying the *IL12RB1*-rs425648 GT or TT genotype (OR = 0.6, 95% CI = 0.3–1.4, $p=0.252$). When exclusive breastfeeding duration was analyzed, we found similar gene-environment interactions not only for rs425648 in the *IL12RB1* gene ($p_{FDR}=0.04$), but also for SNPs in the *TLR9* (rs352140) and *TSLP* genes (rs3806933) (Table 4) ($p_{FDR} < 0.05$). In particular, exclusive breastfeeding duration significantly increased the risk of FS in a dose-responsive manner in children carrying the *TLR9*-rs352140 TT genotype, with ORs of 3.3 (95% CI = 1.0–10.9) and 13.2 (95% CI = 3.0–57.3) for breastfed <4 months and breastfed ≥ 4 months, respectively. A similar trend also was detected in children with the rs352140 CT genotype, but the effect was moderate at best, while no association was found in children with the rs352140 CC genotype (Table 4).

Interactions between the number of unfavorable genotypes and breastfeeding

We then tested the interaction between breastfeeding and the number of unfavorable genotypes for the three identified SNPs shown above. The unfavorable genotype (GG for rs425648, CT/TT for rs3806933 and T-allele for rs352140) was defined as the one(s) for which breastfeeding increased the risk of FS. With zero or one unfavorable genotype possible for rs425648 or rs3806933 based on the best-fitting dominant genetic model, and with zero, one or two unfavorable alleles possible for rs352140 based on the best-fitting additive genetic model, the total number of unfavorable genotypes for each subject was calculated, which potentially ranged from 0 to 4. As shown in Figure 2, breastfeeding tended to be associated with a decreased risk of FS in children carrying zero unfavorable genotypes; this association was attenuated with an increasing number of unfavorable genotypes, and then was significantly reversed in children carrying 3 or more unfavorable genotypes. This led to a strengthened gene-breastfeeding interaction (p for interaction = 7.5×10^{-6}) and gene-exclusive breastfeeding duration interaction (p for interaction = 1.4×10^{-7}) on FS (Figure 2).

We then defined FS as sIgE ≥ 0.70 kU/L to any of eight food allergens and repeated the above analyses. Similar associations between breastfeeding and FS, and similar interactions between breastfeeding and rs425648, rs3806933 and rs352140 were detected (data not shown).

Discussion

In our inner-city US prospective birth cohort (predominantly African American), we found that breastfeeding was independently associated with an increased risk of FS in the total population, especially in children ≥ 2 years. Most importantly, we are the first to report that this positive association was dependent on functional genetic variants in the *IL12RB1*, *TLR9*

and *TSLP* genes, suggesting significant gene-breastfeeding interactions on FS. Our study underscores the importance of evaluating the effects of breastfeeding in the context of individual genetic backgrounds.

A few studies have proposed that the positive association observed between breastfeeding and allergic phenotypes may be due to reverse causation^{14, 15}. Full exploration of this issue requires an investigation of the relationship between early signs of allergy and the decision to commence breastfeeding and/or to stop breastfeeding. In this study, three early signs of allergy, including family history of allergy, detectable vs. non-detectable CBIgE and allergic disease in the first four months of life, were analyzed. None of these variables showed significant associations with never vs. ever breastfeeding and/or with exclusive breastfeeding duration. Our stratified analyses also indicated that the positive association between breastfeeding (or exclusive duration) and FS did not vary by any of these variables. Thus, we believe that the issue of reverse causation, if it exists, was minimized in this study.

Our results showed that breastfeeding may have dual effects on FS by way of increasing risk in children carrying the rs425648 GG genotype but protecting children carrying rs425648 GT/TT genotypes. The apparent promoting effect of breastfeeding in the total population may be due to the fact that children with rs425648 GG genotype outnumbered those with GT/TT genotypes by a factor of 2.9. SNP rs425648 is located in an eQTL of the *IL12RB1* gene, according to SCAN (<http://scan.bsd.uchicago.edu/newinterface/about.html>). The protein encoded by the *IL12RB1* gene is part of the receptor for two IFN- γ -inducing cytokines: IL-12³⁰ and IL-23³¹, which can induce T_H1 responses. By using the SNP genotyping and gene expression data sets from Merck³², we found that the rs425648 T-allele was associated with a decreased *IL12RB1* gene expression in the liver ($p=2\times 10^{-8}$, unpublished data). Consistently, a previous study showed that the rs393548 A-allele in the *IL12RB1* gene, which is in high LD with rs425648 T-allele in Caucasians, could negatively affect *IL12RB1* gene activation and then lead to increased T_H2 skewing³³. Taken together, we propose that breastfeeding may reduce the risk of FS in children with relatively increased T_H2 differentiation (due to decreased *IL12RB1* gene expression), but possibly increase the risk in those with decreased T_H2 differentiation (due to increased *IL12RB1* gene expression). However, the underlying mechanisms are unknown.

Further related to T-cell differentiation, SNP rs3806933, another SNP that showed interaction with breastfeeding, is located in the promoter region of the *TSLP* gene, which triggers dendritic cell-mediated T_H2 inflammatory responses. In our cohort, breastfeeding showed a promoting effect on FS only in children carrying the rs3806933 CT/TT genotype. By again applying the SNP genotyping and gene expression data sets from Merck, we found that the rs3806933 T-allele is associated with a lower expression of the *TSLP* gene in the liver ($p\text{-value}=4\times 10^{-8}$, unpublished data), thus possibly leading to decreased T_H2 differentiation. If this result can be validated in lymphocytes, it would support our hypothesis that breastfeeding may promote the development of FS only in children with relatively decreased T_H2 differentiation.

The hygiene hypothesis has been proposed as one of the mechanisms underlying the allergy-promoting effects of breastfeeding. In detail, breastfeeding is proposed to protect against viral infection, which may induce a skew in the immune system to a T_H2-dominated profile, and thereby, increase the risk of allergic diseases. Interestingly, a third SNP that showed interaction with breastfeeding in this study is a synonymous SNP (rs352140) in the *TLR9* gene, which is involved in pathogen recognition and activation of innate immunity. We found a positive association between breastfeeding and FS only in children carrying the rs352140 T-allele. According to Pupasuite (<http://pupasuite.bioinfo.cipf.es/>), rs352140 may affect exonic splicing regulation and then protein translation. Soriano-Sarabia et al. reported

that the rs352140 T-allele was associated with a decreased CD4 count and an increased viral load³⁴, which may potentially lead to a decreased T_H2 profile. This finding also indicates that breastfeeding may promote the development of FS in children with relatively decreased T_H2 differentiation.

Our tests for interaction between breastfeeding and the combined genotypes of variants in the *IL12RB1* (rs425648), *TLR9* (rs352140) and *TSLP* (rs3806933) genes appeared to be even more convincing than the individual tests, indicating that these three functional SNPs may jointly interact with breastfeeding to affect the risk of FS. This finding is not surprising due to their direct or indirect roles in regulating T_H1/T_H2 balance. However, functional studies on these identified SNPs are needed.

The strengths of our study include its prospective nature and population-based design. We also acknowledge that our study has some limitations. First, misclassification of feeding history due to recall bias cannot be completely avoided. However, this misclassification should be independent of FS status and would not significantly change our results. Second, in this study, FS status was defined only at one time point and this time point varied across children. The classification of FS in early childhood may vary by the age when FS was defined. However, this issue is unlikely to change our findings, since the age when FS was defined was independent of breastfeeding status in this cohort (Table 1). Our data also showed that the positive association between breastfeeding and FS was unchanged in a subset of children with relatively more stable/persistent FS status (that is, FS status defined at ≥ 2 years). Third, only 18 well-known candidate genes involved in T_H1/T_H2 balance and/or innate immunity were analyzed in this study, which might omit other unknown but important genes from the gene-breastfeeding interactions. Finally, it should be noted that food sensitization as measured in this study is often part of a biological response to an allergen, but that alone does not necessarily mean a clinical reaction to a food, therefore, caution is needed when extending our findings to food allergy.

In conclusion, this study suggests that significant gene-breastfeeding interactions be involved in the development of food sensitization. Given the undoubted health benefits of breastfeeding, our findings should not lead to a change in the general recommendations for breast-feeding. Rather our findings should lead to further studies, which would allow for a better understanding of the effect of breastfeeding on allergic diseases in the context of individual genetic variation. The type of information yielded by such studies may enable physicians to provide more personalized medical care and advice to mothers in the future, with the potential to decrease the risk of allergic disease in their children.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AIMs	ancestry informative markers
CBIGe	cord blood IgE
FOXP3	forkhead box P3
GATA3	GATA binding protein
IgE	Immunoglobulin E
IL	Interleukin

<i>IL4R</i>	IL4 receptor
<i>IL12RB1</i>	IL12 receptor, beta1
<i>IL13RA1</i>	IL13 receptor, alpha1
LD	linkage disequilibrium
SNP	single nucleotide polymorphism
<i>STAT6</i>	signal transducer and activator of transcription 6
<i>TBX21</i>	t-box 21
<i>TGFB</i>	transforming growth factor, beta1
T_H	T-helper
T-reg	T-regulatory
<i>TSLP</i>	thymic stromal lymphopoietin
FS	food sensitization
<i>TLR</i>	toll-like receptor
eQTL	expression quantitative trait locus
OR	odds ratio
CI	confidence interval

Key Messages

The relationship between breastfeeding and food sensitization varied significantly by individual genotypes in our study. These findings may help explain inconsistent relationships between breastfeeding and allergic disease reported in the literature.

Figure 1. Plot for the association between breastfeeding and food sensitization in 970 children from the Boston Birth Cohort, stratified by early signs of allergy

* $p < 0.05$, # $p < 0.10$ for the stratified associations between breast-feeding and food sensitization, adjusted by maternal age at delivery, maternal education, maternal smoking during pregnancy, maternal smoking after delivery, children's age, gender, ancestral proportion, and pets in the 1st year.

Figure 2. Gene-environment interaction effect between breastfeeding and the number of unfavorable genotypes of the *IL12RB1*(rs425648), *TSLP*(rs3806933) and *TLR9*(rs352140) genetic variants on food sensitization in 970 children from the Boston Birth Cohort

The unfavorable genotype is defined as the one(s) for which breastfeeding increased the risk of food sensitization, which is GG for rs425648, CT/TT for rs3806933 and T-allele for rs352140.

Table 1

Population characteristics of 970 children from the Boston Birth Cohort, stratified by breastfeeding status.

Variable	Never breast-fed (n=231)	Ever breast-fed (n=739)	p ^a
	Mean±SD		
Maternal age, yrs	27.7±6.4	28.9±6.2	0.01
Maternal BMI, kg/m ²	26.8±6.0	26.6±6.4	0.66
Children's age, yrs	2.5±2.3	2.6±2.2	0.73
	N (%)		
Maternal smoker during pregnancy	56 (24.2)	40 (5.4)	<0.001
Maternal smoker after delivery	91 (39.4)	77 (10.4)	<0.001
Maternal race			<0.001
African American	133 (57.6)	439 (59.4)	
Caucasian	31 (13.4)	26 (3.5)	
Hispanic	39 (16.9)	186 (25.2)	
Others	28 (12.1)	88 (11.9)	
Maternal education			0.001
Primary or secondary	88 (38.1)	215 (29.1)	
High school	88 (38.1)	257 (34.8)	
College or above	55 (23.8)	267 (36.1)	
Household income at visits			0.11
<30K	110 (47.6)	324 (43.8)	
≥30K	22 (9.5)	110 (14.9)	
Unknown	99 (42.9)	305 (41.3)	
Maternal history of allergy, yes	95 (41.1)	255 (34.5)	0.19
Paternal history of allergy, yes	45 (19.5)	123 (16.6)	0.45
Family history of allergy, yes	118 (51.1)	330 (44.7)	0.23
Children's gender, boy %	124 (53.7)	362 (49.0)	0.24
Preterm birth	65 (28.1)	189 (25.6)	0.49
Parity, first born	90 (39.0)	304 (41.1)	0.71
Cesarean section	80 (34.6)	236 (31.9)	0.62
Pets in the first year, yes	47 (20.3)	112 (15.2)	0.13
Detectable cord blood total IgE ^b	119 (51.5)	398 (53.9)	0.68
Allergy in the first 4 months ^c	43 (18.6)	121 (16.4)	0.71

^a t-test and chi-square test were performed for continuous and categorical variables, respectively.^b 40 never breast-fed subjects and 133 ever breast-fed subjects have no information on cord blood total IgE level.^c 9 never breast-fed subjects and 32 ever breast-fed subjects have no information on allergy in the first 4 months.

Table 2

Association between breastfeeding and food sensitization in 970 children from the Boston Birth Cohort.

Variables	n	FS, %	Crude			Adjusted <i>a</i>		
			OR	95%CI	p-value	OR	95%CI	p-value
All children								
Breastfeeding								
Never breast-fed	231	29.4	ref			ref		
Ever breast-fed	739	39.6	1.6	1.1-2.2	0.005	1.5	1.1-2.1	0.019
Exclusive breastfeeding duration <i>b</i>								
Never breast-fed	231	29.4	ref			ref		
<4 months	484	38.4	1.5	1.1-2.1	0.019	1.5	1.0-2.2	0.034
≥4 months	200	40.4	1.6	1.1-2.4	0.022	1.6	1.1-2.5	0.029
Children < 2 years								
Breastfeeding								
Never breast-fed	139	30.2	ref			ref		
Ever breast-fed	414	36.2	1.3	0.9-2.0	0.198	1.1	0.7-1.8	0.616
Exclusive breastfeeding duration <i>b</i>								
Never breast-fed	139	30.2	ref					
<4 months	291	35.7	1.3	0.8-2.0	0.259	1.1	0.7-1.8	0.743
≥4 months	97	36.1	1.3	0.8-2.3	0.345	1.2	0.7-2.2	0.573
Children ≥ 2 years								
Breastfeeding								
Never breast-fed	92	28.3	ref			ref		
Ever breast-fed	325	44.0	2.0	1.2-3.3	0.007	2.3	1.3-4.1	0.003
Exclusive breastfeeding duration <i>b</i>								
Never breast-fed	92	28.3	ref			ref		
<4 months	193	42.5	1.9	1.1-3.2	0.022	2.4	1.3-4.3	0.005
≥4 months	103	43.7	2.0	1.1-3.6	0.026	2.6	1.3-5.0	0.006

Definition of abbreviations: FS = food sensitization; OR = odds ratio; CI = confidence interval

^a Adjusted by maternal age at delivery, family history of allergy, maternal education, maternal smoking during pregnancy, maternal smoking after delivery, children's age when FS was defined, gender, ancestral proportion, pets in the 1st year and allergy during the first four months of life.

^b 55 ever breast-fed children have missing data on exclusive breastfeeding duration.

Table 3

Gene-breastfeeding interactions on food sensitization in 970 children from the Boston Birth Cohort.

SNP <i>a</i>	Genotype	N (FS, %)		BF-FS Association <i>b</i>		P for interaction
		Not BF	BF	OR (95%CI)	p-value	
Interleukin 12 receptor, beta 1 gene (<i>IL12RBI</i>)						
rs425648	GT+TT	57 (40.4)	189 (31.7)	0.6 (0.3-1.4)	0.252	0.0007&
	GG	174 (25.9)	550 (42.4)	2.0 (1.4-3.1)	0.0005	
Interleukin 13 receptor, alpha gene (<i>IL13RA1</i>)						
rs2495637	AA	29 (48.3)	60 (26.7)	0.2 (0.1-0.8)	0.026	0.004
	GG+GA	202 (26.7)	678 (40.9)	1.8 (1.2-2.6)	0.003	
rs2495619	TT+CT	46 (43.5)	123 (38.2)	0.7 (0.3-1.6)	0.433	0.035
	CC	185 (25.9)	614 (39.9)	1.9 (1.3-2.8)	0.002	
Thymic stromal lymphopoietin gene (<i>TSLP</i>)						
rs3806933	CC	109 (36.7)	342 (37.7)	0.9 (0.6-1.5)	0.777	0.006
	CT+TT	121 (23.1)	396 (41.4)	2.4 (1.4-4.0)	0.0008	
Toll-like receptor 9 gene (<i>TLR9</i>)						
rs352140	CC	83 (36.1)	337 (35.9)	0.9 (0.5-1.6)	0.812	0.012
	CT	108 (27.8)	296 (41.2)	2.0 (1.2-3.4)	0.012	
	TT	40 (20.0)	106 (47.2)	4.7 (1.5-14.9)	0.009	
Interleukin-4 gene (<i>IL4</i>)						
rs2243250	CT+CC	150 (32.0)	467(34.7)	1.1 (0.7-1.7)	0.692	
	TT	80 (25.0)	268 (48.5)	2.9 (1.5-5.4)	0.001	0.022

Definition of abbreviations: SNP = single nucleotide polymorphism; BF = breast-fed; FS = Food sensitization; OR = odds ratio; CI = confidence interval.

^a SNPs with unadjusted p for interaction <0.05 were shown.

^b Adjusted by maternal age at delivery, family history of allergy, maternal education, maternal smoking during pregnancy, children's age, gender, ancestral proportion, pets in the 1st year and allergy in the first 4 months of life.

[&] FDR-corrected p < 0.05.

Table 4

Gene-environment interaction between exclusive breastfeeding duration and *IL12RB1*, *TLR9* and *TSLP* SNPs on food sensitization in 970 children from the Boston Birth Cohort.

Interleukin 12 receptor, beta 1 gene (IL12RB1)						
rs425648=GG			rs425648=GT/TT			
Duration ^a	N (FS,%)	OR (95%CI) ^b	N (FS,%)	OR (95%CI) ^b	P _{interaction}	
Never BF	174 (25.9)	ref	57 (40.4)	ref	0.0007&	
<4 months	358 (40.2)	1.9 (1.3-3.0)**	126 (33.3)	0.7 (0.3-1.5)		
≥ 4 months	153 (43.8)	2.4 (1.5-4.0)***	47 (27.7)	0.4 (0.2-1.1)		
Thymic stromal lymphopoietin gene (TSLP)						
rs3806933=CC			rs3806933=CT/TT			
Duration ^a	N (FS,%)	OR (95%CI) ^b	N (FS,%)	OR (95%CI) ^b	P _{interaction}	
Never BF	109 (36.7)	ref	121 (23.1)	ref	0.001&	
<4 months	226 (38.1)	1.0 (0.6-1.7)	258 (38.8)	2.2 (1.3-3.7)**		
≥ 4 months	91 (33.0)	0.7 (0.4-1.4)	108 (46.3)	3.1 (1.7-5.8)***		
Toll-like receptor 9 gene (TLR9)						
rs352140=CC			rs352140=CT			
Duration ^a	N (FS,%)	OR (95%CI) ^b	N (FS,%)	OR (95%CI) ^b	P _{interaction}	
Never BF	83 (36.1)	ref	108 (27.8)	ref	0.0007&	
<4 months	227 (37.9)	1.1 (0.6-2.0)	187 (39.0)	1.7 (1.0-3.1)	3.3 (1.0-10.9)	
≥ 4 months	85 (30.6)	0.8 (0.4-1.5)	84 (40.5)	2.1 (1.0-4.0)*	13.2 (3.0-57.3)***	

Definition of abbreviations: FS = food sensitization; OR = odds ratio; CI = confidence interval.

^a Representing exclusive breast-feeding duration.

^b Adjusted by maternal age at delivery, family history of allergy, maternal education, maternal smoking during pregnancy, maternal smoking after delivery, children's age, gender, ancestral proportion, pets in the 1st year and allergy in the first 4 months of life.

* Nominal p<0.05,

** p<0.01,

p<0.001.
& FDR-corrected p < 0.05.