

Early-life environmental exposures interact with genetic susceptibility variants in pediatric patients with eosinophilic esophagitis

Elizabeth T. Jensen, MPH, PhD,^{a,b} Jonathan T. Kuhl, BS,^c Lisa J. Martin, PhD,^{d,e} Carl D. Langefeld, PhD,^f
Evan S. Dellon, MD, MPH,^{b,g} and Marc E. Rothenberg, MD, PhD^{c,d}

Winston-Salem and Chapel Hill, NC, and Cincinnati, Ohio

Background: Although eosinophilic esophagitis (EoE) is associated with certain gene variants, the rapidly increasing incidence of EoE suggests that environmental factors contribute to disease development.

Objective: We tested for gene-environment interaction between EoE-predisposing polymorphisms (within *TSLP*, *LOC283710/KLF13*, *CAPN14*, *CCL26*, and *TGFB*) and implicated early-life factors (antibiotic use in infancy, cesarean delivery, breast-feeding, neonatal intensive care unit [NICU] admission, and absence of pets in the home).

Methods: We conducted a case-control study using hospital-based cases (n = 127) and control subjects representative of the hospital catchment area (n = 121). We computed case-only interaction tests and in secondary analyses evaluated the combined and independent effects of genotype and environmental factors on the risk of EoE.

Results: Case-only analyses identified interactions between rs6736278 (*CAPN14*) and breast-feeding ($P = .02$) and rs17815905 (*LOC283710/KLF13*) and NICU admission ($P = .02$) but not with any of the factors examined. Case-control analyses suggested that disease risk might be modifiable in subjects with certain gene variants. In particular, breast-feeding in those with the susceptibility gene variant at rs6736278 (*CAPN14*) reduced the risk of EoE (adjusted odds ratio, 0.08; 95% CI, 0.01-0.59). Admission to the NICU in those without the susceptibility gene variant at rs17815905 (*LOC283710/KLF13*) significantly increased the risk of having disease (adjusted odds ratio, 4.83; 95% CI, 1.49-15.66).

Conclusions: The interplay of gene (*CAPN14* and *LOC283710/KLF13*) and early-life environment factors (breast-feeding and NICU admission) might contribute to EoE susceptibility. (J Allergy Clin Immunol 2017;■■■:■■■-■■■.)

Key words: Early-life factors, eosinophilic esophagitis, gene-environment interaction, exposures

From the Departments of ^aEpidemiology and Prevention and ^bBiostatistical Sciences, Wake Forest University Public Health Sciences, Winston-Salem; ^cthe Division of Gastroenterology and Hepatology, Department of Medicine, and ^dthe Center for Esophageal Diseases and Swallowing, University of North Carolina School of Medicine, Chapel Hill; the Divisions of ^eAllergy and Immunology and ^fHuman Genetics, Cincinnati Children's Hospital Medical Center; and ^gthe Department of Pediatrics, University of Cincinnati School of Medicine.

Supported by the American College of Gastroenterology Clinical Research Award. This work was also supported by National Institutes of Health grants R01 DK101856 (to E.S.D.), R37 AI045898, and R01 AI124355; the Campaign Urging Research for Eosinophilic Disease (CURED); the Buckeye Foundation; and the Sunshine Charitable Foundation and its supporters, Denise A. Bunning and David G. Bunning (to M.E.R.).

Disclosure of potential conflict of interest: E. T. Jensen's institution received a grant from the American College of Gastroenterology for this work and grants from the National Institutes of Health (NIH) for other works. E. S. Dellon's institution received a grant from the American College of Gastroenterology for this work and grants from Meritage, Miraca, Nutricia, Receptos, Regeneron, and Shire for other works and an educational grant from Banner and personally received consultancy fees from Adare, Alivio, Banner, Enumeral, GlaxoSmithKline, Receptos, Regeneron, and Shire. M. E. Rothenberg's institution received a grant from the NIH for this work, a US-Israel Binational Grant, and a grant from the PCORI and patent, inventorship, ownership by Cincinnati Children's for other works; personally received consultancy fees from AstraZeneca, Merck, Novartis, Celgene, Genentech, PulmOne Therapeutics, and GlaxoSmithKline; received payment for lectures from Merck; received stock options from PulmOne Therapeutics, NKT Therapeutics, and Immune Pharmaceuticals. The rest of the other authors declare that they have no relevant conflicts of interest.

Received for publication January 11, 2017; revised June 28, 2017; accepted for publication July 10, 2017.

Corresponding author: Elizabeth T. Jensen, MPH, PhD, Wake Forest University School of Medicine, Division of Public Health Sciences, Department of Epidemiology & Prevention, Medical Center Boulevard, Winston-Salem, NC 27157. E-mail: ejensen@wakehealth.edu. Or: Marc E. Rothenberg, MD, PhD, Division of Allergy and Immunology, Cincinnati Center for Eosinophilic Disorders, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, MLC 7028, Cincinnati, OH 45229-3039. E-mail: Rothenberg@cchmc.org.

0091-6749/\$36.00

© 2017 American Academy of Allergy, Asthma & Immunology
<http://dx.doi.org/10.1016/j.jaci.2017.07.010>

Over the past 2 decades, eosinophilic esophagitis (EoE) has transformed from a case-reportable disease to a substantial cause of upper gastrointestinal morbidity.¹ The reasons for this increase are likely multifactorial, including increased disease recognition and changing environmental factors, particularly those interacting with immunologic pathways involved in patients with EoE.^{2,3} Numerous genetic susceptibility variants have been shown to contribute to EoE, including variants at 5q22 (*TSLP/WDR36*), 2p23 (*CAPN14*), and 11q13 (*LRCC32*), but the magnitude of association for disease susceptibility is modest (<2-fold), similar to the magnitude seen in other allergic and immunologic diseases. Twin studies have established a role for genetics in disease susceptibility but an even stronger role for environmental factors, particularly those encountered in early life, as shown by the marked increased concordance for EoE between dizygotic twins compared with nontwin siblings and environmental assessments in EoE case-control studies.^{4,5}

Identification of gene-environment interactions for EoE offers an opportunity to improve understanding of the mechanisms for disease development. It also might offer a means for identifying possible modifiable risk factors for disease, specifically risk factors that might only be relevant in the presence of specific genetic susceptibility variants. Therefore, we conducted a preliminary case-control study of gene-environment interaction between EoE genetic variants⁶⁻⁹ and early-life exposures that have been associated with EoE and other atopic diseases (see Table E1 in this article's Online Repository at www.jacionline.org).^{5,10-12} Specifically, we examined interaction between 5 gene variants (rs6736278 within *CAPN14* at 2p23, rs2302009

Abbreviations used

aOR: Adjusted odds ratio
 EoE: Eosinophilic esophagitis
 NICU: Neonatal intensive care unit
 RERI: Relative excess risk due to interaction
 SNP: Single-nucleotide polymorphism

within *CCL26* at 7q11, rs3806932 within *TSLP* at 5q22, rs17815905 within the *LOC283710* and *KLF13* region at 15q13, and rs1800469 within *TGFB* at 19q13) and 6 maternally reported early-life exposures (cesarean delivery, preterm delivery, neonatal intensive care unit [NICU] admission, breast-feeding, antibiotics in infancy, and absence of a furry pet in infancy).

We selected single-nucleotide polymorphisms (SNPs) reported to be associated with EoE or in high linkage disequilibrium with SNPs associated with EoE. Specifically, these included SNPs from genes with demonstrated functionality in gene regulation and association with allergic sensitization (*TSLP* at 5q22 [rs3806932], the *LOC283710* and *KLF13* region at 15q13 [rs4329885], *CAPN14* [rs6736278], *CCL26* [rs2302009], and *TGFB* [rs1800469]).⁶⁻⁹ *CAPN14* encodes an IL-13–induced, calcium-activated cysteine protease involved in maintaining integrity of esophageal barrier function and is overexpressed in patients with EoE.^{13,14} The rs4329885 SNP, which is found on the 15q13 chromosome band between *LOC283710* and *KLF13*, is in a region that is influential for gene regulation, might contribute to the impaired esophageal epithelial cell development that has been observed to occur in patients with EoE,¹⁵ and has been shown to be involved in T_H2 cytokine production.¹⁶ *TSLP*, a gene that is critical to inducing allergen sensitization, is strongly associated with and overexpressed in patients with EoE.^{9,17} *CCL26* encodes for eotaxin-3, the most upregulated gene in patients with EoE, and acts as a chemoattractant for the activation and recruitment of eosinophils into esophageal tissue.⁸ TGF- β secretion, which is produced in part by eosinophils, is implicated in the fibrosis of esophageal tissue observed in patients with EoE, as well as in immunomodulation.¹⁸ Although the exact mechanism for a possible interaction is unknown, it might be that environmental factors have epigenetic effects that influence DNA expression.^{19,20}

METHODS

Cases were recruited from the Cincinnati Center for Eosinophilic Disorders at Cincinnati Children's Hospital Medical Center and met the criteria for EoE diagnosis, as defined by current consensus guidelines.²¹ Control subjects, which were used to assess violation of the assumption of independence between exposure and genotype in the noncases, were recruited from the Cincinnati Children's Hospital Medical Center Genomic Control Cohort, a population-based control cohort representative of the Greater Cincinnati population.²² Cases and control subjects were restricted to those less than 18 years of age at the time of enrollment, and all were of self-reported white race.

Early-life environmental factor assessment

Early-life environmental factors for cases and control subjects were assessed through an online self-administered questionnaire provided to mothers. The questionnaire, which was described previously in detail,¹⁰ was developed to study early-life exposures through an interactive process that

included cognitive interviewing and testing. Survey domains included prenatal, intrapartum, and pediatric exposures, and the exposures of interest for this study were limited to those previously associated with EoE or demonstrating main effect associations in the current study population,⁵ including cesarean delivery, preterm birth, admission to the NICU, formula feeding, and antibiotic use (see Table E1).^{5,10-12,23}

SNP genotyping and quality control

DNA from cases and control subjects was obtained from peripheral blood samples. Cases and control subjects were genotyped with the genome-wide OMNI5 BeadChip array (Illumina, San Diego, Calif).²² Examination of call rates identified less than 0.1% missing genotypes. We identified no evidence of departure from Hardy-Weinberg equilibrium in cases, control subjects, or cases and control subjects combined (see Table E2 in this article's Online Repository at www.jacionline.org). Before analyses, we performed principal components analysis with EIGENSOFT²⁴ on 639 ancestry informative markers from the OMNI5 BeadChip (Illumina).²²

Statistical analyses

In our primary analysis, we conducted a case-only gene-environment interaction analysis. We used a generalized linear model (logit link and binomial distribution) to assess the association between each environmental factor and genotype in cases. We modeled the risk allele by using a recessive mode of inheritance if the risk allele was the major allele (eg, GG or GA vs GG) and a dominant mode of inheritance if the risk allele was the minor allele (eg, AA vs AG or GG). If there was marginal evidence of an interactive effect ($P < .10$) in the case-only analysis, we tested the assumption of independence between the exposure and genotype in the source population by modeling the association between exposure and genotype in the controls. Lack of independence was identified by evidence of a similar direction and magnitude of association between exposure and genotype in cases and control subjects.

In secondary analyses to facilitate interpretation of any interactive effect, we used cases and control subjects to estimate the odds of disease in those with the susceptibility genotype only, those with the environmental factor only, and those with both the susceptibility genotype and the environmental factor relative to those with neither the susceptibility genotype nor the environmental factor. Additionally, we modeled disease risk within those with the susceptibility genotype as a means for assessing the contribution of each early-life factor in those with the susceptibility genotype. For all models, we estimated both crude and adjusted (adjusted for maternal education and 2 principal components for ancestry) associations. The presence of interaction was determined by indication of a case-only interaction ($P < .05$) and indication of stratum-specific differences in the magnitude of association in the case-control assessment. Finally, we calculated the relative excess risk due to interaction (RERI) to assess for interaction on the additive scale.^{25,26} We generated both crude and adjusted estimates for both primary and secondary analyses. Adjusted models included adjustment for maternal education as a proxy for socioeconomic status and population stratification. The study was approved by the Cincinnati Children's Hospital Medical Center institutional review board.

RESULTS

Of the 237 patients successfully contacted, 169 (71%) consented to participate. Of these, 136 (80%) completed the questionnaire. Similarly, for control subjects, of the 208 potential subjects successfully contacted, 147 (71%) consented to participate, and of these, 125 (85%) completed the questionnaire. Our final analytic sample, after exclusion of observations with missing data, included 127 cases and 121 control subjects. The demographic features of the cases and control subjects were similar, with the exception of atopic disease comorbidities (eg, 83.3% cases vs 12.4% control subjects reporting food allergies) and sex

TABLE I. Characteristics of the study population

Characteristic	Control subjects (n = 121 [% or mean SD])	Cases (n = 127 [% or mean SD])
Age at enrollment (y), mean (SD)	13.8 (2.8)	10.6 (3.8)
Sex (% male)	52.1	80.3
Atopic illnesses (%)		
Food allergies	12.4	83.3
Environmental allergies	42.2	69.3
Antibiotic allergies	14.5	25.8
Eczema	21.5	59.1
Asthma	18.2	44.9
Maternal marital status (%)		
Married or civil union	93.4	94.4
Single	6.6	5.6
Maternal education (%)		
Less than high school	0	2.4
High school or GED	17.4	11.8
Technical or Associates' degree	21.5	18.9
Bachelor's degree	40.5	37.0
Graduate or professional	20.7	29.9

(80.3% male subjects among cases vs 52.1% among control subjects, [Table I](#)).

In our primary case-only analyses, we observed interaction between rs6736278 within *CAPN14* and breast-feeding ($P = .02$, [Fig 1](#)). We also observed interaction between rs17815905 in the *LOC283710* and *KLF13* region and NICU admission ($P = .02$). There was no evidence of a statistically significant interaction between the SNPs in *CCL26*, *TSLP*, or *TGFB* with any of the early-life factors examined (see [Table E3](#) in this article's Online Repository at www.jacionline.org). Where there was evidence of interaction or marginal evidence of interaction (*TSLP* and NICU admission and *TSLP* and no furred pets in infancy), we found no evidence of violation of independence (see [Table E4](#) in this article's Online Repository at www.jacionline.org). Secondary analyses confirmed the case-only interactions observed ([Fig 2](#) and [Tables II](#) and [III](#)), and stratum-specific assessments identified breast-feeding as a potentially modifiable factor in development of EoE among children with a certain *CAPN14* genotype ([Table II](#)). Notably, we observed a 92% reduction in the odds of having EoE among those who were breast-fed within the subgroup of participants harboring the susceptibility genotype ([Table II](#)). Examination of interaction on the additive scale, RERI, identified a strong departure from additivity for *CAPN14* and breast-feeding (RERI, -6.27 ; 95% CI, -17.27 to 4.72 ; [Table II](#)) and the *LOC283710* and *KLF13* region and NICU admission (RERI, -4.53 ; 95% CI, -10.81 to 1.76); however, neither of these reached statistical significance ([Table III](#)).

DISCUSSION

In this study, we examined interaction between 5 gene variants (rs6736278 within *CAPN14* at 2p23, rs2302009 within *CCL26* at 7q11, rs3806932 within *TSLP* at 5q22, rs17815905 within the *LOC283710* and *KLF13* region at 15q13, and rs1800469 within *TGFB* at 19q13) and 6 maternally reported early-life exposures (cesarean delivery, preterm delivery, NICU admission, breast-feeding, antibiotics in infancy, and absence of a furry pet in infancy). We conducted case-only tests of interaction

(with subsequent confirmation of results with the case-control analysis) and observed evidence of statistical interaction between rs6736278 (*CAPN14*) and breast-feeding and rs17815905 (*LOC283710* and *KLF13* region) and NICU admission. Case-control analyses of these associations provided additional evidence to support the presence of an interaction. The combined effect of susceptibility genotype and environmental exposure was less than expected (eg, antagonistic) after examining the independent effect of genotype and environmental exposure. For example, the combined effect for breast-feeding and genotype was significantly less (adjusted odds ratio [aOR], 0.43; 95% CI, 0.12-1.62) than the effect of genotype (aOR, 5.38; 95% CI, 0.89-32.71) or breast-feeding alone (aOR, 1.70; 95% CI, 0.76-3.77). Indeed, breast-feeding had a strong protective effect (aOR, 0.08; 95% CI, 0.01-0.59) in those with the susceptibility genotype (rs6736278 within *CAPN14*). Although the exact mechanism of how breast-feeding and *CAPN14* can interact and become protective for disease is unknown, the protective effect of breast-feeding was demonstrated in a study of interaction between breast-feeding and the CD14 genotype (CD14C-159T) in relation to atopic sensitization.²⁷ *CAPN14* is integral to maintaining barrier function in the esophagus.^{13,14} Potentially, with impaired barrier function, breast-feeding might confer a greater protective effect than that observed when barrier function is preserved; however, this must be investigated in follow-up mechanistic studies.

The early-life factors examined in this study have been associated with dysbiosis in gut colonization and early life.²⁸⁻³⁶ Indeed, the esophagus harbors its own microbiome that is dysregulated in patients with EoE.³⁷ It is interesting to speculate that the interacting SNPs might mediate their effects by modifying esophageal microbiota content and/or their interaction with EoE-related immunologic pathways, even before EoE onset.^{28,38-40} Although this study was not designed to directly assess the influence of these exposures on the microbiome, the results provide the first potential evidence of gene-environment interactions in patients with EoE and support future work examining the microbiome and EoE.

Our results must be interpreted with caution because this preliminary study had a relatively small sample size, and we have not sought to replicate these findings in an independent sample. Furthermore, the associations reported required only nominal significance ($P < .05$) and did not account for multiple testing. To try to minimize the risk of false-positive results, we limited the analyses and environmental effects that had been associated with EoE risk in prior studies. Given that many of the exposures are correlated (eg, preterm delivery and NICU admission), each exposure-genotype interaction assessment did not represent a completely independent test, and the exposures and SNPs selected were selected *a priori* based on hypotheses of an interactive effect, as opposed to an agnostic study of gene-environment interaction in which multiple correction testing might be more relevant.⁴¹ Although we observed interaction for several of the environmental exposures examined, these exposures might be a proxy for some other factor or factors that are interacting with the identified genes. Additionally, although early-life factors, such as cesarean delivery, breast-feeding, NICU admission, and having pets in the home, are likely not hampered by recall bias, recall of antibiotic use and possibly preterm delivery could be biased.

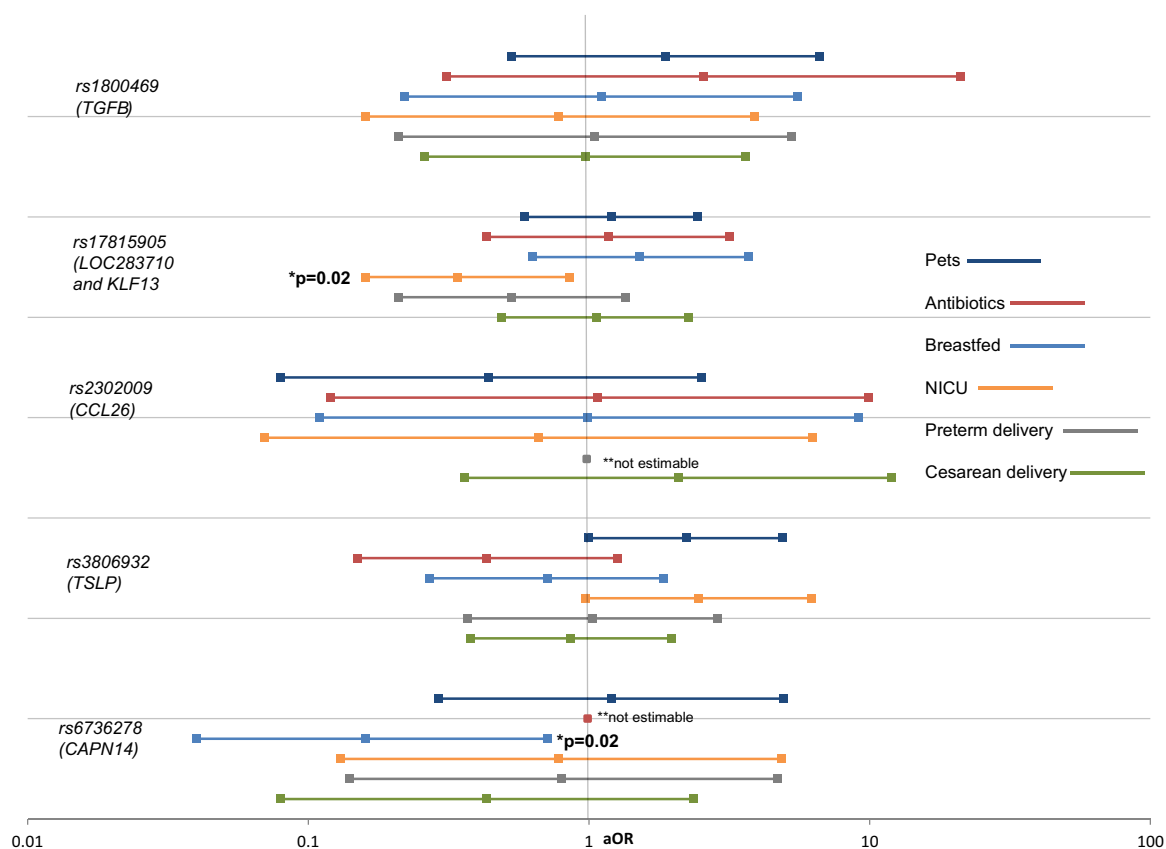


FIG 1. Case-only assessment of gene-environment interaction in patients with EoE. The illustration shows association between exposure and genotype in cases only to assess for gene-environment interaction adjusted for maternal education. *Suggestive associations examined in case-control data assessment of interaction. **Data are too sparse for estimation of association.

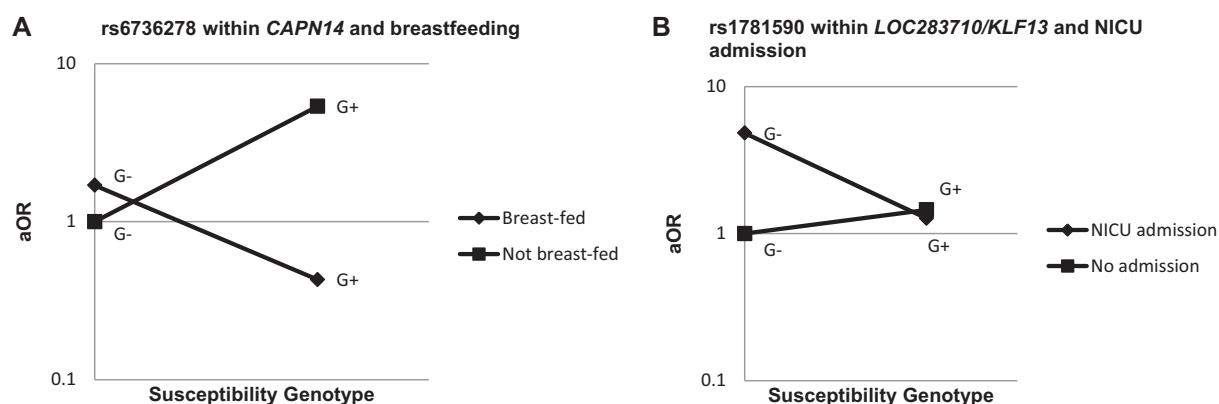


FIG 2. Case-control analysis interaction plots for early-life factors and susceptibility genotype. Plots denote interaction between selected exposures and genotype variants in the case-control analyses. Interaction models include adjustment for maternal education. G+, Exposed to susceptibility genotype variant; G-, unexposed to susceptibility genotype variant. **A**, Difference in risk for those breast-fed and not breast-fed among those with the susceptibility genotype variant. **B**, Increase in risk for NICU admission and those without the susceptibility variant.

In conclusion, we have preliminary evidence that the cause of EoE might involve the interplay of gene and environmental factors. Further understanding of these interactions provides an

opportunity to potentially alter the development of EoE because environmental factors can be modified in subjects harboring specific genetic variants.

TABLE II. *CAPN14* (rs6736278) and breast-feeding

	Cases (no.)	Control subjects (no.)	OR (95% CI)	aOR (95% CI)
Interaction				
G−E−	26	11	Referent	Referent
G+E−	2	5	5.91 (0.99-35.21)	5.38 (0.89-32.71)
G−E+	75	60	1.89 (0.86-4.14)	1.70 (0.76-3.77)
G+E+	18	4	0.53 (0.14-1.91)	0.43 (0.12-1.62)
Case-only <i>P</i> value for interaction			.01	.02
RERI (95% CI)			−6.27 (−17.27 to 4.72)	
Effect of exposure among those with the susceptibility genotype				
G+E−	2	5	Referent	Referent
G+E+	18	4	0.09 (0.01-0.63)	0.08 (0.01-0.59)

TABLE III. *LOC283710* and *KLF13* region (rs17815905) and NICU admission

	Cases (no.)	Control subjects (no.)	OR (95% CI)	aOR (95% CI)
Interaction				
G−E−	38	51	Referent	Referent
G+E−	63	56	1.47 (0.85-2.55)	1.45 (0.83-2.53)
G−E+	16	4	5.23 (1.62-16.89)	4.83 (1.49-15.66)
G+E+	9	10	1.18 (0.44-3.17)	1.27 (0.46-3.53)
Case-only <i>P</i> value for interaction			.02	.02
RERI (95% CI)			−4.53 (−10.81 to 1.76)	
Effect of exposure among those with susceptibility genotype				
G+E−	63	56	Referent	Referent
G+E+	9	10	0.80 (0.30-2.11)	0.88 (0.32-2.38)

Clinical implications: EoE-predisposing polymorphisms interact with early-life factors to modify the risk of EoE disease development. The risk for EoE disease might be modifiable in subjects with certain environmental exposures and gene variants.

REFERENCES

- Dellon ES, Gonsalves N, Hirano I, Furuta GT, Liacouras CA, Katzka DA. ACG clinical guideline: evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). *Am J Gastroenterol* 2013;108:679-92.
- Hruz P, Bussmann C, Heer P, Simon HU, Zwahlen M, Beglinger C, et al. Escalating epidemiology of eosinophilic esophagitis: 21 years of prospective population-based documentation in Olten County. *Gastroenterology* 2011; 140(suppl 1):S238-9.
- Dellon ES, Erichsen R, Baron JA, Shaheen NJ, Vyberg M, Sorensen HT, et al. The increasing incidence and prevalence of eosinophilic esophagitis outpaces changes in endoscopic and biopsy practice: national population-based estimates from Denmark. *Aliment Pharmacol Ther* 2015;41:662-70.
- Alexander ES, Martin LJ, Collins MH, Kottyan LC, Sucharew H, He H, et al. Twin and family studies reveal strong environmental and weaker genetic cues explaining heritability of eosinophilic esophagitis. *J Allergy Clin Immunol* 2014;134: 1084-92.e1.
- Jensen ET, Kuhl JT, Martin LJ, Rothenberg ME, Dellon ES. Prenatal, intrapartum, and postnatal factors are associated with pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* 2017 [Epub ahead of print].
- Sherrill JD, Rothenberg ME. Genetic dissection of eosinophilic esophagitis provides insight into disease pathogenesis and treatment strategies. *J Allergy Clin Immunol* 2011;128:23-34.
- Collins MH, Blanchard C, Abonia JP, Kirby C, Akers R, Wang N, et al. Clinical, pathologic, and molecular characterization of familial eosinophilic esophagitis compared with sporadic cases. *Clin Gastroenterol Hepatol* 2008;6:621-9.
- Blanchard C, Wang N, Stringer KF, Mishra A, Fulkerson PC, Abonia JP, et al. Eo-tatin-3 and a uniquely conserved gene-expression profile in eosinophilic esophagitis. *J Clin Invest* 2006;116:536-47.
- Rothenberg ME, Spergel JM, Sherrill JD, Annaiah K, Martin LJ, Cianferoni A, et al. Common variants at 5q22 associate with pediatric eosinophilic esophagitis. *Nat Genet* 2010;42:289-91.
- Jensen ET, Kappelman MD, Kim HP, Ringel-Kulka T, Dellon ES. Early life exposures as risk factors for pediatric eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2013;57:67-71.
- Radano MC, Yuan Q, Katz A, Fleming JT, Kubala S, Shreffler W, et al. Cesarean section and antibiotic use found to be associated with eosinophilic esophagitis. *J Allergy Clin Immunol Pract* 2014;2:475-7.e1.
- Slae M, Persad R, Leung A-T, Gabr R, Brooks D, Huynh H. Role of environmental factors in the development of pediatric eosinophilic esophagitis. *Dig Dis Sci* 2015; 60:3364-72.
- Davis BP, Stucke EM, Khorki ME, Litosh VA, Rymer JK, Rochman M, et al. Eosinophilic esophagitis-linked calpain 14 is an IL-13-induced protease that mediates esophageal epithelial barrier impairment. *JCI Insight* 2016;1: e86355.
- Litosh VA, Rochman M, Rymer JK, Porollo A, Kottyan LC, Rothenberg ME. Calpain-14 and its association with eosinophilic esophagitis. *J Allergy Clin Immunol* 2017;139:1762-71.e7.
- Rochman M, Travers J, Miracle CE, Bedard MC, Wen T, Azouz NP, et al. Profound loss of esophageal tissue differentiation in patients with eosinophilic esophagitis. *J Allergy Clin Immunol* 2017 [Epub ahead of print].
- Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The Human Genome Browser at UCSC. *Genome Res* 2002;12: 996-1006.
- Sherrill JD, Gao PS, Stucke EM, Blanchard C, Collins MH, Putnam PE, et al. Variants of thymic stromal lymphopoietin and its receptor associate with eosinophilic esophagitis. *J Allergy Clin Immunol* 2010; 126:160-5.e3.
- Rieder F, Nonevski I, Ma J, Ouyang Z, West G, Protheroe C, et al. T-helper 2 cytokines, transforming growth factor beta1, and eosinophil products induce fibrogenesis and alter muscle motility in patients with eosinophilic esophagitis. *Gastroenterology* 2014;146:1266-77, e1-9.
- Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, et al. Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res* 2012;22:1790-7.
- Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012;40(Database issue):D930-4.
- Liacouras CA, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, et al. Eosinophilic esophagitis: Updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 2011;128:3-20.e6.
- Kottyan LC, Davis BP, Sherrill JD, Liu K, Rochman M, Kaufman K, et al. Genome-wide association analysis of eosinophilic esophagitis provides insight into the tissue specificity of this allergic disease. *Nat Genet* 2014; 46:895-900.
- Price AL, Neves JF, Nurko S, Olszak T, Exley MA, Blumberg RS, et al. Involvement of the iNKT cell pathway is associated with early-onset eosinophilic esophagitis and response to allergen avoidance therapy. *Am J Gastroenterol* 2014; 109:646-57.
- Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006;38:904-9.
- Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. *Int J Epidemiol* 2012;41:514-20.
- Knol MJ, VanderWeele TJ, Groenwold RH, Klungel OH, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. *Eur J Epidemiol* 2011;26:433-8.
- Lee SY, Kang MJ, Kwon JW, Park KS, Hong SJ. Breastfeeding might have protective effects on atopy in children with the CD14C-159T CT/CC genotype. *Allergy Asthma Immunol Res* 2013;5:239-41.
- Huurre A, Kalliomaki M, Rautava S, Rinne M, Salminen S, Isolauri E. Mode of delivery—effects on gut microbiota and humoral immunity. *Neonatology* 2008; 93:236-40.
- Mulder IE, Schmidt B, Lewis M, Delday M, Stokes CR, Bailey M, et al. Restricting microbial exposure in early life negates the immune benefits associated with gut colonization in environments of high microbial diversity. *PLoS One* 2011;6: e28279.
- Patel AL, Mutlu EA, Sun Y, Koenig L, Green S, Jakubowicz A, et al. Longitudinal survey of microbiota in hospitalized preterm very low birth weight infants. *J Pediatr Gastroenterol Nutr* 2016;62:292-303.

31. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO Rep* 2012;13:440-7.
32. Taft DH, Ambalavanan N, Schibler KR, Yu Z, Newburg DS, Ward DV, et al. Intestinal microbiota of preterm infants differ over time and between hospitals. *Microbiome* 2014;2:36.
33. Tormo-Badia N, Hakansson A, Vasudevan K, Molin G, Ahme S, Cilio CM. Antibiotic treatment of pregnant non-obese diabetic mice leads to altered gut microbiota and intestinal immunological changes in the offspring. *Scand J Immunol* 2014;80:250-60.
34. van Nimwegen FA, Penders J, Stobberingh EE, Postma DS, Koppelman GH, Kerkhof M, et al. Mode and place of delivery, gastrointestinal microbiota, and their influence on asthma and atopy. *J Allergy Clin Immunol* 2011;128:948-55, e1-3.
35. Walker WA, Iyengar RS. Breast milk, microbiota, and intestinal immune homeostasis. *Pediatr Res* 2015;77:220-8.
36. Westerbeek EA, van den Berg A, Lafeber HN, Knol J, Fetter WP, van Elburg RM. The intestinal bacterial colonisation in preterm infants: a review of the literature. *Clin Nutr* 2006;25:361-8.
37. Harris JK, Fang R, Wagner BD, Choe HN, Kelly CJ, Schroeder S, et al. Esophageal microbiome in eosinophilic esophagitis. *PLoS One* 2015;10:e0128346.
38. Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* 2012;336:489-93.
39. Walker A. Intestinal colonization and programming of the intestinal immune response. *J Clin Gastroenterol* 2014;48(suppl 1):S8-11.
40. Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosh D, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med* 2016;22:1187-91.
41. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1:43-6.

REFERENCES

- E1. Jensen ET, Kappelman MD, Kim HP, Ringel-Kulka T, Dellon ES. Early life exposures as risk factors for pediatric eosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2013;57:67-71.
- E2. Radano MC, Yuan Q, Katz A, Fleming JT, Kubala S, Shreffler W, et al. Cesarean section and antibiotic use found to be associated with eosinophilic esophagitis. *J Allergy Clin Immunol Pract* 2014;2:475-7.e1.
- E3. Slae M, Persad R, Leung AJ, Gabr R, Brocks D, Huynh HQ. Role of environmental factors in the development of pediatric eosinophilic esophagitis. *Dig Dis Sci* 2015;60:3364-72.
- E4. Jensen ET, Kuhl JT, Martin LJ, Rothenberg ME, Dellon ES. Prenatal, intrapartum, and postnatal factors are associated with pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* 2017 Jun 7 [Epub ahead of print].

TABLE E1. Previous studies evaluating the association between early-life factors and EoE

Study	Study population	No. of subjects	Early-life factor	Direction of association
Jensen et al, 2013 ^{E1}	Hospital-based cases and control subjects (non-GI and GI*)	31 cases, 26 control subjects (age, 1-17 y)	Antibiotic use	+
			Cesarean delivery	+
			Breast-feeding (exclusive)	—
			Maternal smoking/postnatal ETS	No association (maternal)
			Acid suppressants	Not reported
			Pets in home	Not reported
Radano et al, 2014 ^{E2}	Hospital-based cases and control subjects (non-GI)	25 cases, 74 control subjects (age, 1-5 y)	Antibiotic use	+
			Cesarean delivery	+
			Breast-feeding (any)	No association
			Maternal smoking/postnatal ETS	Not reported
			Acid suppressants	Not reported
			Pets in home	Not reported
Slæe et al, 2015 ^{E3}	Hospital-based cases and control subjects (GI functional disease control subjects)	102 cases, 167 control subjects (age, 1-18 y)	Antibiotic use	No association
			Cesarean delivery	Not reported
			Breast-feeding (exclusive)	No association
			Maternal smoking/postnatal ETS	— (Postnatal exposure)
			Acid suppressants	Not reported
			Pets in home	No association
Jensen et al, 2016 ^{E4}	Hospital-based cases and population-based control subjects	127 cases, 121 control subjects (age, 1-18 y)	Antibiotic use	+
			Cesarean delivery	+
			Breast-feeding (exclusive)	No association
			Maternal smoking/postnatal ETS	No association (maternal)
			Acid suppressants	+
			Pets in home	—

ETS, Environmental tobacco smoke; GI, gastrointestinal.

*GI control subjects were functional disease controls (gastroesophageal reflux disease) and were identified to not adequately represent the underlying source population giving rise to the cases.

TABLE E2. Quality control and minor allele frequencies of study SNPs

Chromosome	Gene	SNP	Minor allele	Missing rate*	HWE			MAF	
					All	Patients with EoE	Control subjects	Patients with EoE	Control subjects
2	<i>CAPN14</i>	rs6736278	A	0.0007968	0.6509	1	0.7615	0.0778	0.0616
5	<i>TSLP</i>	rs3806932	G	0.0007968	0.5596	0.1808	1	0.3563	0.4391
7	<i>CCL26</i>	rs2302009	C	0.0007968	0.4047	0.3782	0.7761	0.2489	0.2469
15	<i>LOC283710</i> and <i>KLF13</i> region	rs17815905	G	0	0.3504	0.5642	0.5785	0.2056	0.2559
19	<i>TGF-β</i>	rs1800469	A	0	0.4692	0.5171	0.6786	0.32	0.3062

HWE, Hardy-Weinberg equilibrium; *MAF*, minor allele frequency.

*Missing rate = proportion of alleles that were not genotyped.

TABLE E3. Case-only assessment of gene-environment interaction

	<i>CAPN14</i> rs6736278 (n = 80)		<i>TSLP</i> rs3806932 (n = 126)		<i>CCL26</i> rs2302009 (n = 126)		<i>LOC283710</i> and <i>KLF13</i> region rs17815905 (n = 126)		<i>TGFβ</i> rs1800469 (n = 126)	
	Crude OR (95% CI)	aOR (95% CI)	Crude OR (95% CI)	aOR (95% CI)	Crude OR (95% CI)	aOR (95% CI)	Crude OR (95% CI)	aOR (95% CI)	Crude OR (95% CI)	aOR (95% CI)
Cesarean delivery										
No	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Yes	0.53 (0.10-2.72) <i>P</i> = .44	0.43 (0.08-2.36) <i>P</i> = .33	0.92 (0.42-2.00) <i>P</i> = .83	0.86 (0.38-1.97) <i>P</i> = .73	1.95 (0.38-10.10) <i>P</i> = .43	2.08 (0.36-11.95) <i>P</i> = .41	1.01 (0.48-2.11) <i>P</i> = .98	1.06 (0.49-2.26) <i>P</i> = .89	0.94 (0.27-3.31) <i>P</i> = .92	0.97 (0.26-3.61) <i>P</i> = .96
Preterm delivery										
No	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Yes	1.07 (0.20-5.68) <i>P</i> = .94	0.80 (0.14-4.68) <i>P</i> = .81	0.86 (0.33-2.29) <i>P</i> = .77	1.03 (0.37-2.88) <i>P</i> = .95	Not estimable	Not estimable	0.52 (0.21-1.30) <i>P</i> = .16	0.53 (0.21-1.35) <i>P</i> = .18	0.90 (0.18-4.39) <i>P</i> = .89	1.05 (0.21-5.29) <i>P</i> = .95
NICU admission										
No	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Yes	1.07 (0.20-5.68) <i>P</i> = .94	0.78 (0.13-4.84) <i>P</i> = .79	2.73 (1.11-6.67) <i>P</i> = .03	2.46 (0.97-6.23) <i>P</i> = .06†	0.81 (0.09-7.25) <i>P</i> = .85	0.66 (0.07-6.26) <i>P</i> = .72	0.35 (0.14-0.86) <i>P</i> = .02	0.34 (0.13-0.85) <i>P</i> = .02†	0.80 (0.16-3.91) <i>P</i> = .78	0.78 (0.16-3.90) <i>P</i> = .76
Breast-fed										
No	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Yes	0.15 (0.03-0.63) <i>P</i> = .01	0.16 (0.04-0.71) <i>P</i> = .02†	0.85 (0.34-2.13) <i>P</i> = .73	0.71 (0.27-1.85) <i>P</i> = .48	0.99 (0.11-9.16) <i>P</i> = .99	0.99 (0.11-9.16) <i>P</i> = .99	1.55 (0.64-3.72) <i>P</i> = .33	1.52 (0.63-3.70) <i>P</i> = .36	1.25 (0.26-6.10) <i>P</i> = .78	1.11 (0.22-5.52) <i>P</i> = .78
Antibiotics in infancy*										
No	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
Yes	Not estimable	Not estimable	0.47 (0.17-1.28) <i>P</i> = .14	0.43 (0.15-1.26) <i>P</i> = .12	1.08 (0.12-9.85) <i>P</i> = .94	1.07 (0.12-9.93) <i>P</i> = .95	1.18 (0.44-3.20) <i>P</i> = .74	1.17 (0.43-3.17) <i>P</i> = .76	2.57 (0.31-21.21) <i>P</i> = .38	2.55 (0.31-21.14) <i>P</i> = .38
Cats or dogs in infancy										
Yes	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent	Referent
No	1.22 (0.30-4.90) <i>P</i> = .78	1.20 (0.29-4.93) <i>P</i> = .80	1.93 (0.90-4.14) <i>P</i> = .09	2.22 (1.00-4.91) <i>P</i> = .05†	0.42 (0.07-2.36) <i>P</i> = .32	0.44 (0.08-2.53) <i>P</i> = .36	1.21 (0.60-2.44) <i>P</i> = .60	1.20 (0.59-2.43) <i>P</i> = .64	1.83 (0.52-6.43) <i>P</i> = .34	1.88 (0.53-6.63) <i>P</i> = .33

OR, Odds ratio.

*Twenty-one had missing data for characterizing antibiotic use in infancy.

†Assumption of independence tested (Table E4).

TABLE E4. Test of independence assumption for case-only design*

	<i>CAPN14</i> and breast-feeding, aOR (95% CI)	<i>TSLP</i> and NICU admission, aOR (95% CI)	<i>TSLP</i> and no furry pets, aOR (95% CI)	<i>LOC283710</i> and <i>KLF13</i> region and NICU admission, aOR (95% CI)
Association in cases	0.16 (0.04-0.71); $P = .02$	2.46 (0.97-6.23); $P = .06$	2.22 (1.00-4.91); $P = .05$	0.34 (0.13-0.85); $P = .02$
Association in control subjects	2.22 (0.46-10.67); $P = .32$	0.74 (0.41-1.34); $P = .89$	0.80 (0.37-1.77); $P = .59$	1.98 (0.56-7.00); $P = .29$

*For those with P values of .10 or less in the case-only test of interaction, models include adjustment for maternal education and population stratification.