Twin and family studies reveal strong environmental and weaker genetic cues explaining heritability of eosinophilic esophagitis

Eileen S. Alexander, MS\textsuperscript{a,b,c}, Lisa J. Martin, PhD\textsuperscript{a,b}, Margaret H. Collins, MD\textsuperscript{a,b}, Leah Kottyan, PhD\textsuperscript{b}, Heidi Sucharew, PhD\textsuperscript{b}, Hua He, MS\textsuperscript{b}, Vincent A. Mukkada, MD\textsuperscript{a,b}, Paul A. Succop, PhD\textsuperscript{a}, J. Pablo Abonia, MD\textsuperscript{a,b}, Heather Foote\textsuperscript{b}, Michael D. Eby, BS\textsuperscript{b}, Tommie M. Grotjan, BS\textsuperscript{b}, Alexandria J. Greenler, BS\textsuperscript{b}, Evan S. Dellow, MD, MPH\textsuperscript{d}, Jeffrey G. Demain, MD\textsuperscript{b}, Glenn T. Furuta, MD\textsuperscript{i}, Larry E. Gurian, MD, AGAF\textsuperscript{g}, John B. Harley, MD, PhD\textsuperscript{a,b,h}, Russell J. Hopp, DO\textsuperscript{j}, Ajay Kaul, MD\textsuperscript{a,b}, Kari C. Nadeau, MD, PhD\textsuperscript{k}, Richard J. Noel, MD, PhD\textsuperscript{m}, Philip E. Putnam, MD\textsuperscript{a,b}, Karl F. von Tiehl, MD\textsuperscript{n}, and Marc E. Rothenberg, MD, PhD\textsuperscript{a,b}

\textsuperscript{a}University of Cincinnati College of Medicine, Departments of Environmental Health, Pediatrics, Pathology and Laboratory Medicine, Cincinnati, OH

\textsuperscript{b}Cincinnati Children's Hospital Medical Center, Divisions of Biostatistics and Epidemiology; Human Genetics; Pathology; Rheumatology, Center for Autoimmune Genomics and Etiology; Gastroenterology, Hepatology and Nutrition; Allergy and Immunology, Cincinnati Center for Eosinophilic Disorders, Cincinnati, OH

\textsuperscript{c}Xavier University, Health Services Administration, Cincinnati, OH

\textsuperscript{d}University of North Carolina School of Medicine, Division of Gastroenterology and Hepatology, Center for Esophageal Diseases and Swallowing, Chapel Hill, NC

\textsuperscript{e}Director, Allergy, Asthma and Immunology Center of Alaska, Anchorage, AK

\textsuperscript{f}Children's Hospital Colorado, Digestive Health Institute, Gastrointestinal Eosinophilic Diseases Program, University of Colorado School of Medicine, Aurora, CO

\textsuperscript{g}Ferrell Duncan Clinic and CoxHealth, Springfield, MO

\textsuperscript{h}U.S. Department of Veterans Affairs Medical Center, Cincinnati, OH

\textsuperscript{i}Department of Pediatrics, Division of Allergy and Immunology, Creighton University, Omaha, NE

\textsuperscript{j}Stanford Medical School, Stanford, CA

\textsuperscript{k}Stanford Medical Center and Lucille Packard Children's Hospital, Division of Allergy and Immunology, Stanford, CA

\textsuperscript{l}Children’s Hospital of Wisconsin, Milwaukee, WI

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Correspondence: Marc E. Rothenberg, MD, PhD, Cincinnati Children's Hospital Medical Center, Division of Allergy and Immunology, MLC 7028, 3333 Burnet Avenue, Cincinnati, OH 45229. rothenberg@cchmc.org.

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Abstract

Background—Eosinophilic esophagitis (EoE) is a chronic antigen-driven allergic inflammatory disease, likely involving the interplay of genetic and environmental factors, yet their respective contributions to heritability are unknown.

Objective—To quantify risk associated with genes and environment on familial clustering of EoE.

Methods—Family history was obtained from a hospital-based cohort of 914 EoE probands, (n=2192 first-degree “Nuclear-Family” relatives) and the new international registry of monozygotic and dizygotic twins/triplets (n=63 EoE “Twins” probands). Frequencies, recurrence risk ratios (RRRs), heritability and twin concordance were estimated. Environmental exposures were preliminarily examined.

Results—Analysis of the Nuclear-Family–based cohort revealed that the rate of EoE, in first-degree relatives of a proband, was 1.8% (unadjusted) and 2.3% (sex-adjusted). RRRs ranged from 10–64, depending on the family relationship, and were higher in brothers (64.0; p=0.04), fathers (42.9; p=0.004) and males (50.7; p<0.001) compared to sisters, mothers and females, respectively. Risk of EoE for other siblings was 2.4%. In the Nuclear-Families, combined gene and common environment heritability ($h^2_{ge}$) was 72.0±2.7% (p<0.001). In the Twins cohort, genetic heritability was 14.5±4.0% (p<0.001), and common family environment contributed 81.0±4% (p<0.001) to phenotypic variance. Proband-wise concordance in MZ co-twins was 57.9±9.5% compared to 36.4±9.3% in DZ (p=0.11). Greater birth-weight difference between twins (p=0.01), breastfeeding (p=0.15) and Fall birth season (p=0.02) were associated with twin discordance in disease status.

Conclusions—EoE recurrence risk ratios are increased 10–64-fold compared with the general population. EoE in relatives is 1.8–2.4%, depending upon relationship and sex. Nuclear-Family heritability appeared to be high (72.0%). However, Twins cohort analysis revealed a powerful role for common environment (81.0%) compared with additive genetic heritability (14.5%).

Keywords
eosinophilia; medical genetics; twins; immune system diseases; heritability; gene-environment interaction; drug hypersensitivity; gastrointestinal diseases; skin diseases

Introduction

Eosinophilic esophagitis (EoE) is a debilitating, chronic allergic inflammatory disease of the esophagus triggered by food and ingested antigen sensitization followed by T helper type 2 (Th2) cell adaptive immune responses. Although EoE prevalence has increased in both adult\textsuperscript{1–4} and pediatric populations,\textsuperscript{5,6} strategies for prevention, management and risk mitigation are limited.\textsuperscript{7} Research on underlying biologic processes has resulted in new opportunities for treatment, yet risk factors for EoE remain unclear.
One mechanism for high EoE risk is genetic variation. Indeed, Blanchard, et al., estimated an 80-fold increase in sibling recurrence risk, compared to population prevalence, suggesting a strong genetic component.\(^8\) The importance of genetic variants is supported by both candidate gene and genome-wide association studies.\(^9\) Genetic variants in \(\text{CAPN14}, \text{TSLP}, \text{TSLPR}, \text{CCL26}, \text{and FLG}\) have been associated with EoE.\(^{10-13}\) However, these variants explain only a small portion of EoE cases, leaving a large portion of the variation unexplained.

There is also substantial evidence that environmental factors influence EoE risk. First and foremost, EoE is an allergic condition responsive to allergen exposure via respiratory, gastrointestinal or cutaneous routes.\(^{14-17}\) For example, EoE is induced in murine models via respiratory exposure of \(\text{Aspergillus fumigatus}\) antigens,\(^{16}\) and molds, including \(\text{Aspergillus}\) and \(\text{Penicillium}\), are associated with eosinophilic asthma.\(^{18}\) Recently, early environmental exposures, such as antibiotic exposure in the first year of life,\(^{19}\) have been implicated. Indeed, birth season, climate, seasonality\(^{20-24}\) and \(\text{Helicobacter pylori}\) exposure\(^{25,26}\) modify disease susceptibility. Further, epigenetic regulation\(^{27,28}\) may play a role in altered expression\(^{29-31}\) associated with EoE. Despite these intriguing findings, the relative roles of genetic and environmental factors in EoE risk are unclear.

The purpose of this study was to estimate the contributions of genes and environment to EoE risk in susceptible families. To accomplish this objective, we used a cohort of nuclear families at the Cincinnati Center for Eosinophilic Disorders (CCED) at Cincinnati Children’s Hospital Medical Center (CCHMC) and established a new cohort with histologically confirmed EoE in at least one twin/triplet.

**METHODS**

To quantify EoE risk due to genes and environment in familial clustering, a retrospective cross-sectional study was conducted using the Nuclear-Family cohort derived from the CCED database and the newly created EoE Twins Registry. The study was performed with CCHMC IRB approval and review by the University of Cincinnati IRB. Participants or their parent/guardians provided written consent. Children over the age of eleven years provided written assent.

The CCED database was used for the period of August 1, 2008 to April 30, 2013 to identify patients and collect basic demographics, clinical testing and family history. Probands were identified by their CCED physician. Additional history of related medical conditions for first-degree relatives was obtained by parent-report or self-report, using pre-visit questionnaire with subsequent physician confirmation, available in CCHMC’s electronic medical record. Family medical conditions included EoE and other eosinophilic gastrointestinal (GI) diseases (EGID), including eosinophilic gastritis, eosinophilic enteritis and eosinophilic colitis. CCED probands missing physician-confirmed family history were excluded. Among the 1366 CCED patients seen during this time period, 914 (69%) were included.
Established in 2008, the EoE Twins Registry is an international twin/triplet cohort for EoE and related eosinophilic conditions and was created for this CCHMC study. Recruitment is from physicians specializing in allergy and gastroenterology, centers specializing in EoE, patient and parent EoE interest foundations and twin social networking groups. Initial screening of potential participants was by self/parent report of EoE and EGID. EoE Twins are from the continental United States (n=57), Alaska (n=2) and Australia (n=4). Information for Twins <18 years of age was provided by parent report.

**Inclusion and Exclusion Criteria**

Eligible participants/parents were asked for reported diagnosis (EoE, other GI conditions, or unaffected). For all participants that reported EoE, esophagogastroduodenoscopy (EGD) pathology report at diagnosis was reviewed. Pathology slides were requested for all participants with esophageal eosinophils and reviewed by a single pathologist at the CCED (MHC) for the area (0.3 mm²) of greatest intraepithelial eosinophil density. Peak counts were generated (100% of Nuclear-Family; 96% of Twins) to confirm ≥15 eosinophils per high-power field (hpf) at 400X magnification. Slides were requested from an endoscopy performed while the participant was receiving therapy with proton pump inhibitors (PPI) but had not received therapy specifically for EoE, such as steroids and/or diet elimination, as recommended in the EoE consensus guidelines. PPI administration prior to a positive endoscopy was confirmed in 52% of Nuclear-Family probands for whom data were available (55%). Affected Twins diagnostic dates ranged from 2001–2012, with 93% diagnosed prior to publication of the current guidelines recommending PPI screening prior to diagnostic endoscopy. Participants with known causes of peripheral blood eosinophilia were excluded. Individuals with reported EoE without confirmatory pathology reports were excluded.

Registry data included demographics (race, ethnicity, sex, age), birth information (gestational age, use of fertility treatments, birth order, birth-weight, birth-length), medical history and family medical history for each family member. Twins were requested to provide a saliva sample for DNA collection; Oragene™ kit (DNA Genotek, Kanata, Ontario, Canada) was used according to manufacturer’s instructions, with sponges added for children unable to expectorate, typically ≤5 years of age, and prepIT™ L2P manual DNA purification protocol.

**Zygosity**

Three tools determined zygosity of same-sex twins as monozygotic (MZ) or dizygotic (DZ): 1) genotyping, 2) pea pod questionnaire and 3) parent report. To genetically determine zygosity, we estimated the proportion of identity-by-descent (IBD) sharing between each pair of genotyped individuals and compared it to the proportion expected based on genealogical information. The percentage of identical markers was determined from 94544 high-quality, polymorphic markers, among 196524 variants genotyped by Immunochip (Illumina, San Diego, CA). MZ pairs have identical markers at more than 99% of loci with observed IBD sharing of 0.99–1.0. Analysis was limited to same-sex pairs (n=48) with paired DNA samples available (n=40). For same-sex pairs without paired DNA samples, pea pod questionnaire determined zygosity. Pea pod questionnaire is a validated
survey designed to determine how alike twins are based on who can tell them apart\textsuperscript{32}, with 96% accuracy relative to genotyping.\textsuperscript{35} Genetic zygosity results were used as the determinant when available.

**Data Management**

Study data were collected and managed using REDCap electronic data capture tools hosted at CCHMC.\textsuperscript{36}

**Environmental Screening**

Because EoE often has an early onset, we focused on perinatal exposures, such as prenatal vitamins, gestational age, breastfeeding and birth-weight, length and order. Birth seasons included winter (northern hemisphere, December 1-March 20), spring (March 21-May 31), summer (June 1-September 20) and autumn (September 21-November 30). Participants from Australia were coded for southern hemisphere birth seasons. Environmental data included food and medication allergies. Data for parent/self-reported factors were obtained from the EGID database for Nuclear-Families and by telephone interview for Twins and their nuclear families. Penicillin, amoxicillin and cephalosporins were grouped together for analysis.

**Statistical Analysis**

Demographic data and EoE risk estimates were analyzed using JMP Genomics 6.0 (SAS Institute, Cary, NC). Reported p-values are two-tailed with significance at p \leq 0.05, unless otherwise specified; exact values at p \leq 0.001 or p<0.001, were confirmed by permutation test for zero cells.

Demographic characteristics were described using mean \pm standard deviation (SD) for normally distributed continuous traits, median and interquartile range for non-normally distributed continuous traits and frequency for discrete traits. Comparability of subgroups was tested using non-parametric Wilcoxon rank sum, parametric t-tests or Chi-square, as appropriate.

**Recurrence Risk Ratios and Concordance Estimates**

Recurrence risk ratios (RRR) were calculated as (number affected/total)/prevalence, with the point estimate for prevalence set at 5.5 per 10,000.\textsuperscript{1-3} Given the male preponderance of EoE, sex-adjusted frequencies and RRR were calculated; prevalence was set at 8.1 for males and 2.8 for females, on the basis of the 74% male proband frequency in the Nuclear-Family cohort. RRR estimates were compared using a goodness of fit test (\chi^2). Proband-wise concordance, which provides an estimate for agreement of disease state between twins while accounting for ascertainment, was calculated as \(2C/(2C+D)\textsuperscript{37}\), where C is the number of concordant pairs and D is the number of discordant pairs.

**Heritability Analyses**

To estimate the proportion of variation attributable to genes (heritability) we used variance components analysis for nuclear families and structural equations modeling for twins. Because genes and common environment are not able to be separated in nuclear families, we
denoted this heritability as combined gene-environment \( h_{ge}^2 \). Details are specified in an Online Supplement.

**EoE and Environment**

EoE risk associated with individual early environmental exposures, such as parent/self-report of penicillin allergy, was analyzed. Concordance and early life environmental exposures were analyzed for paired covariates, such as age. EoE and non-EoE groups were assumed to be independent; correlation between the twin sets was ignored due to small sample size. Non-parametric Wilcoxon rank sum, parametric t-tests or Chi-square were used, as appropriate.

**RESULTS**

**Description of Nuclear-Family and Twin Cohorts**

Of the 6108 individuals in the 1366 nuclear families screened at the CCED, 914 probands had family history available (69%). After excluding grandparents (n=2391) and twin families (n=31), the Nuclear-Family cohort comprised 914 probands and 2192 first-degree relatives (n=3106) (Figure I). Twin recruiting strategies identified 91 interested families, of whom 63 met study inclusion criteria and 73% provided family environmental history. For same-sex pairs, twin zygosity was ascertained with parent report, pea pod questionnaire and genotyping. Of the 40 pairs with both parent report and genotyping, there was 82.5% agreement. Of the 40 pairs with both pea pod and DNA zygosity, there was 95.0% agreement. One same-sex pair had parent report of zygosity only. Importantly, recruitment of twin pairs was random with respect to zygosity and concordance, and age by concordance was not significantly different for MZ vs. DZ pairs (p=0.96). There were no significant differences between MZ and DZ twins with respect to race or ethnicity, but MZ twins were more likely to be male (p<0.001) and older (p=0.006; Table I). There were no significant differences between the Nuclear-Family and Twin cohorts with respect to sex, race, ethnicity or age. The median ages of Nuclear-Family (range 1.0–64.0 years) and Twin (range 3.0–51.8 years) cohort probands were 12.3–13.2 years with interquartile ranges of approximately 7.7 to 19.1 years of age. Interestingly, both cohorts were 73–74% male, 87–94% white and 94% non-Hispanic.

**Frequency, Recurrence and Concordance of EoE**

To characterize familial clustering of EoE, we first calculated EoE frequency in first-degree relatives of probands. Overall, 1.8% of first-degree relatives had EoE (Table II). Given the higher rate of EoE in males, we examined sex-adjusted frequency, which increased to 2.3%. The risk of having another child with EoE was 2.4% in the Nuclear-Family cohort. Fathers (2.4%; p=0.004) and brothers (3.5%; p<0.04) had EoE at significantly higher rates compared to mothers (0.6%) and sisters (1.3%), respectively. EoE frequency in both MZ (41.0%) and DZ (22.0%) twins was significantly higher than in siblings (Figure II). Surprisingly, EoE frequency in DZ twins was increased compared to non-twin siblings from the Nuclear-Family cohort (p<0.001, Figure II).
Compared to the general population, the risk of EoE for first-degree relatives from the Nuclear-Family cohort (n=2192) was increased; RRR (RRR= $\lambda_R$) was highest in brothers (64.0; p=0.04) and fathers (42.9; p=0.004), compared to sisters (24.0) and mothers (9.9), respectively. Males had higher RRR compared to females (50.7 vs. 14.7; p<0.001) (Table II). Sibling RRR compared to parent RRR (44.2 vs. 25.8; p=0.09; Table II) was not significantly higher. Sex-stratified RRRs implicated greatly increased risk for sisters (adj $\lambda_R$=45.5), mothers (adj $\lambda_R$=19.1), and females (adj $\lambda_R$=28.2).

Proband-wise concordance in MZ co-twins was 57.9±9.5% compared to 36.4±9.3% in DZ twins. Although these concordances were not significantly different from each other (p=0.11), the higher rates of EoE in MZ compared to DZ are supportive of genetic patterning.

**Familial Patterning Supports Non-Mendelian and Complex Mode of Inheritance**

Examining familial patterning in more detail, information can be gained about the likely mode of inheritance (Figure III). Traditional Mendelian inheritance includes dominant, recessive, and X-linked patterns. In dominant inheritance, transmission between an affected parent and a child is ~50%; however, in the Nuclear-Family cohort, 98% of probands have unaffected parents. Autosomal recessive inheritance often has children with unaffected parents, but ~25% of probands’ siblings would also be affected. Overall EoE frequency in affected siblings is 2.4%, much less than expected in an autosomal recessive disorder. Only 1.9% of families had at least one additional EoE affected sibling. Lastly, male predominance of EoE creates suspicion for X-linked inheritance. However, parent-to-child transmission was observed from both mothers and fathers, and father-to-son transmission is not supportive of X-linked inheritance. Thus, it is reasonable to deduce that EoE has a complex mode of inheritance.

**Contribution of Genes and Environment to Familial Clustering**

To quantify the effects of genes and environment, we used both the Nuclear-Family and Twin cohorts. In the Nuclear-Family cohort, combined gene-environment “heritability” ($h_{gc}^2$) was estimated at 72% (p<0.001; SE=0.027) of the total phenotypic variance, suggesting a strong affect from genetics. Parallel analyses in twins estimated combined AE “heritability” ($h_{ae}^2$) at 99.5% (p<0.001). However, the model that separates genetic heritability and common environment (ACE, Goodness of fit p=0.56) fit the data better than either the model with genetics (AE, Goodness of fit p<0.001) or common environment (CE, Goodness of fit p=0.006) (Table III), suggesting that EoE risk resulted from both genetic and shared environmental factors. Importantly, the heritability (estimate 14.5±4%; p<0.001; Figure IVA) changed greatly by analysis of twins, when accounting for a common environment component. The reduction in heritability is attributable to the large proportion of variation explained by common environment (estimate 81.0±4.0%; p<0.001; Figure IVA). Thus, heritability estimates are markedly inflated when common environment is not accounted for (Figure IVB).
Evidence for Shared Environmental Effects

Given increased EoE rates in DZ twins compared to non-twin siblings, we tested environmental factors that may be shared between twin pairs but not necessarily between siblings. Although sample size was limited, greater differences in birth-weight were associated with disease discordance in twin pairs (p=0.01; n=35; Table IV). Birth season was significantly different in concordant and discordant twin pairs (p=0.03; n=63); specifically, birth in Fall was associated with EoE discordance (p=0.02; n=63). Food allergies (p<0.001; n=97) were associated with EoE, and penicillin allergies (p=0.17; n=66) and breastfeeding (p=0.15; n=59) may influence risk for EoE.

DISCUSSION

Previous studies reported familial clustering of EoE, suggesting that clustering is attributable to genetics. Indeed, our large cohort of Nuclear-Families demonstrated that family members are at increased EoE risk compared to the general population and that inheritance is complex and not Mendelian. The Nuclear-Family–based design yielded an inflated heritability (proportion of variation explained by genes) estimate. However, our Twins’ heritability estimates suggest that familial clustering is due in large part to common, or shared, family environment rather than genetics. We demonstrated that environmental factors, such as food and parent/self report of penicillin allergies, and greater difference in birth-weight, may affect EoE risk, whereas Fall birth season and breastfeeding may reduce risk, supporting further exploration of early life factors. Thus, we propose that disease susceptibility in genetically pre-disposed families may be potentiated by early life environment. Notably, colonization by immune-shaping commensal microbiota, in the gut and also in the esophagus, could be a key determinant of environmental risk.

First-degree Relatives of Probands have a Higher Rate of EoE than the General Population

In the 1.9% of families in the Nuclear-Family cohort that had at least one additional child with EoE, 2.4% of probands’ siblings also had EoE. This is a 44-fold increase over the general population prevalence and consistent with the previously published high rate. Compared to other allergic diseases, such as asthma with sibling RRR between 1.25 and 2.25, the sibling RRR of EoE is much higher. We also found EoE enrichment in all first-degree relatives of probands, with fathers and brothers being particularly at risk. EoE is likely underestimated in pediatric subgroups. In the Nuclear-Family cohort, the relatively low risk of having at least one additional child who also has EoE (1.9%; Figure III) is not supportive of an autosomal recessive inheritance proportion indicative of carrier parents. Conversely, relatively low parent-to-child transmission (2.0%), observed for both mothers and fathers, does not support autosomal dominant inheritance. Father-to-son transmission refutes traditional Mendelian X-linked inheritance. Therefore, these data collectively support EoE having a non-Mendelian, or complex, pattern of inheritance involving numerous genetic and environmental factors.

Family Studies Reveal Genetic Susceptibility

Enrichment in first-degree relatives, in our study and others, suggests a genetic component, and, indeed, Nuclear-Family heritability was estimated at 72%. A strong
genetic basis for EoE is further supported by candidate and genome-wide association studies that identified risk variants, as well as EoE-specific gene expression profiles. However, estimating heritability from nuclear families has limited interpretation, as genes and family environment cannot be distinguished. Specifically, similar environmental exposures and risk within the common family environment mimic genetic inheritance patterns and confound heritability. Thus, high heritability estimates in nuclear family study designs may be explained in part by common environment, in addition to genetic susceptibility.

Twin, or extended family, study designs disentangle the effects of genes from common environment. Indeed, the heritability estimate from the reduced AE model (h^2; which ignores common environment) was inflated (99.5%). This high value is not unexpected as twin models often produce inflated estimates due to ascertainment bias. However, by including common environment in the full model, heritability is estimated at 14.5%, with common environment accounting for 81.0% of the variation. The importance of common environment is further supported by our finding that DZ twins are enriched for EoE compared to non-twin siblings. Thus, using the traditional nuclear family approach, the proportion of variation expected to be explained by genetic factors is dramatically overestimated. This overestimation is a problem because these heritability-based estimates are often used as a metric for the amount of variation expected to be explained by single-nucleotide polymorphisms in traditional genetic association studies. The failure of single-nucleotide polymorphisms to account for this variation has been termed “missing” heritability, and “phantom” heritability is speculated to be the result of genetic interactions. Our results show that the amount of variation attributed to genetic factors is overestimated due to failure to account for common family environment.

**Early Life Exposures Likely Contribute**

Our results suggest that early life exposures likely contribute to EoE risk. High concordance of EoE for DZ twins compared to non-twin siblings is unexpected because both non-twin siblings and DZ twins share on average 50% of their genome; thus, the inflation of EoE rates in DZ twins is likely not due to genetic factors. Concomitant timing of exposures during specific windows of critical early development may play an important role in EoE pathogenesis. Preliminary family environmental data suggest that factors in early life, such as birth season, breastfeeding, and penicillin allergy, which implies previous antibiotic use, are likely to be important given that these factors are associated with twin concordance for EoE. Indeed, antibiotic use during infancy has recently been identified as a risk factor for EoE. Prior studies and our data substantiate the importance of early life exposures, such as antibiotics, specifically penicillins and cephalosporins, that alter gut colonization, likely reflecting the role of the metagenome and early microbiota and helminth colonization in priming the developing immune system. Parent/self-report of penicillin-like allergies in twins differentiates concordant and discordant pairs. Further, young children ingest food, water, juice, airborne particles, soil, and dust exposure doses many times higher compared to adults, presenting an opportunity for identification of novel environmental risk factors that alter expression at an early age. An environmental affect on EoE risk is plausible given the dynamic nature of the EoE transcriptome, which varies with allergen exposure (e.g. Alexander et al. Page 9 J Allergy Clin Immunol. Author manuscript; available in PMC 2015 November 01.)
Our breastfeeding data suggest a protective effect against EoE, consistent with current recommendations. Although birth-weight differences between twins and birth season may affect outcomes, they are less modifiable. These data should be interpreted with caution given small sample size of the Twin cohort and their first-degree relations.

In summary, we have demonstrated that EoE clusters in families and much of the clustering can be attributed to common family environment. These results are clinically important because our EoE families report considerable concern about EoE risk when planning their family. Evidence-based risk assessment data show that, overall, the risk is modest (2.4%), but does seem to be increased by the presence of affected parents and offspring. Much of this familial clustering is attributable to environmental factors, suggesting that for individuals with a family history of EoE, identification of early life factors will be essential to reduce risk. We propose that early life exposures prime genetically susceptible individuals for the development of EoE, highlighting the need to rigorously identify salient genetic and environmental risk mechanisms. Thus, future prospective clinical studies will facilitate translation of these findings to actionable recommendations.

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**Abbreviations**

- **EoE**: Eosinophilic esophagitis
- **RRR**: recurrence risk ratio
- **h^ag_2**: narrow-sense additive genetic heritability
- **h^ge_2**: combined additive genetic and common environment heritability
- **CCED**: Cincinnati Center for Eosinophilic Disorders
- **CCHMC**: Cincinnati Children’s Hospital Medical Center
- **MZ**: monozygotic
- **DZ**: dizygotic
- **EGID**: eosinophilic gastrointestinal disease
- **EGD**: esophagogastroduodenoscopy
GERD  gastroesophageal reflux disease
VCM  variance components model

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**Clinical Implications**

The risk of having a second child with EoE is 2.4%. Common family environment (81.0%) and additive genetic heritability (14.5%) explain familial clustering. Early environmental modification may lessen EoE risks.
**Figure I.**
Recruitment Algorithms and Case Identification for Nuclear-Family and Twin Cohorts

A. Nuclear-Family Cohort. B. Twin Cohort.

A. Nuclear-Family cohort from the Cincinnati Center for Eosinophilic Disorders; B. EoE Twins International Registry cohort. EGD, esophagogastroduodenoscopy; EoE, eosinophilic esophagitis; Not EoE, unaffected by eosinophilic esophagitis; MZ, monozygotic; DZ, dizygotic.

*Includes 1 MZ discordant triplet
*Includes 1 DZ concordant triplet
Figure II.
Rates of EoE in Twin Cohort and Nuclear-Family Cohort Sibling Non-probands Frequency of EoE in dizygotic (DZ) non-proband co-twins (n=36), non-proband Nuclear-Family siblings of proband (n=782) compared to population prevalence by $X^2_{df=1}$. MZ, monozygotic.
Figure III.
Summary Pedigrees Support a Complex Mode of EoE Inheritance. A. Nuclear Family Cohort. B. Twin Cohort (Monozygotic). C. Twin Cohort (Dizygotic). Diamond shape represents both brothers and sisters whose number range by “Number of probands’ siblings.” Frequency (%) is the percent of families with that summary pedigree as a percent of all families in panels A, B, and C. In the large Nuclear-Family cohort, families with unaffected parents and at least one additional brother or sister with EoE comprise 1.9%.
**Figure IV.**


A. “ACE” latent class path analysis estimates (point prevalence estimate at 5.5/10,000) represent a generalized model across all twins and all families. By convention, latent variables are represented as ovals and measured variables as squares; MZ, monozygotic; DZ, dizygotic.

B. Twin cohort ACE path analysis (black) separates common family environment, estimating heritability at 14.5±4% (p<0.001) with superior model fit (p=0.56). As expected, using the same data and model but excluding common family environment (dark gray)
inflates heritability to 99.5%. Similarly, Nuclear-Family cohort (light gray) inflates heritability estimate to $72 \pm 2.7\%$ (p<0.001; liability threshold model); A, additive genetic variance (heritability); C, common, shared household, environmental variance; E, unique environment “error” variance.
Table I

Demographics of EoE Nuclear-Family and Twin Cohorts

<table>
<thead>
<tr>
<th></th>
<th>Nuclear-Family</th>
<th>Twin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>All</td>
</tr>
<tr>
<td>All Families (n)</td>
<td>914</td>
<td>63</td>
</tr>
<tr>
<td>Male Sex (%)</td>
<td>74.0</td>
<td>73.4</td>
</tr>
<tr>
<td>Race (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>86.7</td>
<td>93.7</td>
</tr>
<tr>
<td>Black</td>
<td>3.9</td>
<td>0</td>
</tr>
<tr>
<td>Asian</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>AI/AN</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>8.4</td>
<td>6.4</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>94.2</td>
<td>93.7</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Missing</td>
<td>3.9</td>
<td>3.2</td>
</tr>
<tr>
<td>Age (years, median) (IQR) Range</td>
<td>12.3 (7.7–17.2)</td>
<td>13.2 (8.1–19.1)</td>
</tr>
</tbody>
</table>

AI/AN, American Indian or Alaska Native; DZ, dizygotic; IQR, interquartile range; MZ, monozygotic; MZ>DZ male sex (*p<0.001). MZ>DZ age (**p=0.006). All others: not significantly different by \(X^2\), Fisher’s Exact, or Wilcoxon nonparametric test.
Table II

Frequency and Recurrence Risk Ratios ($\lambda_R$) in EoE Nuclear-Family Cohort First-degree Relatives

<table>
<thead>
<tr>
<th>First-degree Relative</th>
<th>Frequency (%)</th>
<th>p-value</th>
<th>Sex-adjusted Frequency (%)</th>
<th>RRR</th>
<th>Sex-stratified RRR</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>1.8</td>
<td></td>
<td></td>
<td>32.5</td>
<td>---</td>
</tr>
<tr>
<td>Males</td>
<td>2.8*</td>
<td>&lt;0.001</td>
<td>2.3</td>
<td>50.7*</td>
<td>34.3</td>
</tr>
<tr>
<td>Females</td>
<td>0.8</td>
<td></td>
<td></td>
<td>14.7</td>
<td>28.2</td>
</tr>
<tr>
<td>Parents</td>
<td>1.4</td>
<td></td>
<td></td>
<td>25.8</td>
<td>---</td>
</tr>
<tr>
<td>Fathers</td>
<td>2.4*</td>
<td>0.004</td>
<td>1.9</td>
<td>42.9*</td>
<td>29.0</td>
</tr>
<tr>
<td>Mothers</td>
<td>0.6</td>
<td></td>
<td></td>
<td>9.9</td>
<td>19.1</td>
</tr>
<tr>
<td>Siblings</td>
<td>2.4</td>
<td></td>
<td></td>
<td>44.2</td>
<td>---</td>
</tr>
<tr>
<td>Brothers</td>
<td>3.5*</td>
<td>0.04</td>
<td>2.9</td>
<td>64.0*</td>
<td>43.2</td>
</tr>
<tr>
<td>Sisters</td>
<td>1.3</td>
<td></td>
<td></td>
<td>24.0</td>
<td>45.5</td>
</tr>
</tbody>
</table>

RRR, recurrence risk ratio (frequency/prevalence); Prevalence at 5.5/10,000; Sex-stratified prevalence for males is 8.1 and 2.8 for females on the basis of the 74% male proband frequency. Unadjusted *p<0.05 by $X^2_{df=1}$. 

Table III

Nested ACE Twin Models to Estimate Heritability

<table>
<thead>
<tr>
<th>Model</th>
<th>Twin Pair Intraclass Correlation</th>
<th>Parameter Estimates</th>
<th>Model Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MZ</td>
<td>DZ</td>
<td>(a^2)</td>
</tr>
<tr>
<td>ACE</td>
<td>0.955</td>
<td>0.883</td>
<td>0.145</td>
</tr>
<tr>
<td>CE</td>
<td>0.940</td>
<td>0.940</td>
<td>---</td>
</tr>
<tr>
<td>AE</td>
<td>0.995</td>
<td>0.498</td>
<td>0.995</td>
</tr>
</tbody>
</table>

A (ag) additive genetic; C (c) common environmental exposures; E (e) error due to unique environmental exposures df, degrees of freedom; DZ, dizygotic; MZ, monozygotic; Non-significant p-value for \(\chi^2\) indicates superior fit of the model to the data.
### Table IV

**Preliminary Screen of Environmental and Co-morbid Risk Factors in the Twin Cohort**

#### A. Twin Pair

<table>
<thead>
<tr>
<th>Exposures for Pairs (maximum n=63)</th>
<th>n</th>
<th>Discordant Frequency (% or mean±SD)</th>
<th>Concordant Frequency (% or mean±SD)</th>
<th>p-value</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Age (years)</td>
<td>63</td>
<td>16.3±11.3</td>
<td>16.0±11.8</td>
<td>0.96</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>43</td>
<td>35.0±3.4</td>
<td>35.0±2.2</td>
<td>0.58</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>Pre-term Birth (≥33.5 weeks)</td>
<td>43</td>
<td>75.7</td>
<td>80.0</td>
<td>1.00</td>
<td>1.3</td>
<td>0.2–7.3</td>
</tr>
<tr>
<td>Term Birth (≥35 weeks)</td>
<td>43</td>
<td>50.0</td>
<td>69.7</td>
<td>0.25</td>
<td>0.4</td>
<td>0.1–1.8</td>
</tr>
<tr>
<td>Twin Birth-Weight Difference (grams)</td>
<td>35</td>
<td>335.7±273.0</td>
<td>145.6±133.7</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth Season Adjusted for Hemisphere</td>
<td>63</td>
<td>Fall 43.2% Winter 13.6% Spring 18.2% Summer 25.0%</td>
<td>Fall 10.5% Winter 31.6% Spring 10.5% Summer 47.4%</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth Season Fall</td>
<td>63</td>
<td>43.2%</td>
<td>10.5%</td>
<td>0.02</td>
<td>0.2</td>
<td>0.03–0.8</td>
</tr>
<tr>
<td>Fertility Treatments</td>
<td>47</td>
<td>45.7</td>
<td>33.3%</td>
<td>0.52</td>
<td>0.6</td>
<td>0.2–2.3</td>
</tr>
<tr>
<td>Fertility Treatment (by type)</td>
<td>20</td>
<td>sparse data</td>
<td></td>
<td>0.43</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>Chorion/Amnion Number</td>
<td>32</td>
<td>sparse data</td>
<td></td>
<td>0.56</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>Prenatal Vitamins</td>
<td>44</td>
<td>93.9%</td>
<td>100%</td>
<td>0.99</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>Birth Order (twins only)</td>
<td>45</td>
<td>47.1%</td>
<td>54.6%</td>
<td>0.67</td>
<td>1.4</td>
<td>0.3–5.3</td>
</tr>
<tr>
<td>Penicillin Allergy in Family</td>
<td>44</td>
<td>21.2%</td>
<td>36.4%</td>
<td>0.42</td>
<td>2.1</td>
<td>0.5–9.4</td>
</tr>
</tbody>
</table>

#### B. Individual Twin

<table>
<thead>
<tr>
<th>Individual Twin Exposures (maximum n=128)</th>
<th>n</th>
<th>Not EoE</th>
<th>EoE</th>
<th>p-value</th>
<th>OR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfeeding</td>
<td>59</td>
<td>90.0%</td>
<td>65.3%</td>
<td>0.15</td>
<td>0.2</td>
<td>0.02–1.8</td>
</tr>
<tr>
<td>Birth Order (second, twins only)</td>
<td>91</td>
<td>47.1%</td>
<td>50.1%</td>
<td>0.72</td>
<td>1.2</td>
<td>0.5–2.7</td>
</tr>
<tr>
<td>Birth-weight (grams)</td>
<td>80</td>
<td>2400±663</td>
<td>2358±532</td>
<td>0.77</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Birth-length (inches)</td>
<td>38</td>
<td>19.2±1.4</td>
<td>18.6±1.1</td>
<td>0.24</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Allergies, environmental</td>
<td>97</td>
<td>64.7%</td>
<td>76.2%</td>
<td>0.23</td>
<td>1.7</td>
<td>0.7–4.3</td>
</tr>
<tr>
<td>Allergies, Spring</td>
<td>69</td>
<td>90.9%</td>
<td>83.0%</td>
<td>0.48</td>
<td>0.49</td>
<td>0.09–2.5</td>
</tr>
<tr>
<td>Allergies, Summer</td>
<td>13</td>
<td>66.7%</td>
<td>80.0%</td>
<td>1.00</td>
<td>2.0</td>
<td>0.1–34.8</td>
</tr>
<tr>
<td>Allergies, Fall</td>
<td>68</td>
<td>86.3%</td>
<td>87.0%</td>
<td>1.00</td>
<td>1.1</td>
<td>0.2–4.7</td>
</tr>
</tbody>
</table>
### B. Individual Twin

<table>
<thead>
<tr>
<th>Individual Twin Exposures (maximum n=128)</th>
<th>n</th>
<th>Not EoE</th>
<th>EoE</th>
<th>p-value</th>
<th>OR</th>
<th>CI 95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergies, Winter</td>
<td>66</td>
<td>59.1%</td>
<td>61.4%</td>
<td>0.86</td>
<td>1.1</td>
<td>0.4–3.1</td>
</tr>
<tr>
<td>Allergies, year round</td>
<td>66</td>
<td>61.9%</td>
<td>64.4%</td>
<td>0.84</td>
<td>1.1</td>
<td>0.4–3.3</td>
</tr>
<tr>
<td>Food Allergies</td>
<td>97</td>
<td>23.5%</td>
<td>81.0%</td>
<td>&lt;0.001</td>
<td>13.8</td>
<td>5.0–38.0</td>
</tr>
<tr>
<td>Penicillin Allergy</td>
<td>66</td>
<td>0.0%*</td>
<td>100.0%</td>
<td>0.17</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

CI 95, 95th percentile for confidence interval; OR, odds ratio;

* confirmed by permutation test. Environmental risk exposures for individual twins/triplets (n=128) by EoE-affected status; twin pairs (n=63) by disease concordance for EoE. Pearson correlation or Fisher’s Exact Test was used for discrete variables; Student t-test for continuous variables.