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

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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 the 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 an 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 to 64, depending on the family relationship, and were higher in brothers (64.0; $P = .04$), fathers (42.9; $P = .004$), and males (50.7; $P < .001$) than in sisters, mothers, and females, respectively. The risk of EoE for other siblings was 2.4%. In the Nuclear-Family cohort, combined gene and common environment heritability was 72.0% ± 2.7% ($P < .001$). In the

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Twins cohort, genetic heritability was 14.5% ± 4.0% ($P < .001$), and common family environment contributed 81.0% ± 4% ($P < .001$) to phenotypic variance. Probandwise concordance in monozygotic co-twins was 57.9% ± 9.5% compared with 36.4% ± 9.3% in dizygotic co-twins ($P = .11$). Greater birth weight difference between twins ($P = .01$), breast-feeding ($P = .15$), and fall birth season ($P = .02$) were associated with twin discordance in disease status.

Conclusions: EoE RRRs are increased 10- to 64-fold compared with the general population. EoE in relatives is 1.8% to 2.4%, depending on relationship and sex. Nuclear-Family heritability appeared to be high (72.0%). However, the Twins cohort analysis revealed a powerful role for common environment (81.0%) compared with additive genetic heritability (14.5%). (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Eosinophilia, food allergy, medical genetics, twins, immune system diseases, heritability, gene-environment interaction, drug hypersensitivity, gastrointestinal diseases, skin diseases*

Eosinophilic esophagitis (EoE) is a debilitating, chronic allergic inflammatory disease of the esophagus triggered by food and ingested antigen sensitization followed by T_H2-cell adaptive immune responses. Although the prevalence of EoE has increased in both adult[1-4] and pediatric populations,[5,6] strategies for prevention, management, and risk mitigation are limited.[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[8] estimated an 80-fold increase in recurrence risk in siblings, compared with population prevalence, suggesting a strong genetic component. The importance of genetic variants is supported by both candidate gene and genomewide association studies.[9] Genetic variants in *CAPN14, TSLP, TSLPR, CCL26*, 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 the risk of EoE. 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 to *Aspergillus fumigatus* antigens,[16] and molds, including *Aspergillus* and *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 *Helicobacter pylori* exposure[25,26] modify disease susceptibility. Furthermore, 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 the risk of EoE are unclear.

The purpose of this study was to estimate the contribution of genes and the environment to the risk of EoE 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 1 twin/triplet.

Abbreviations used

CCED:	Cincinnati Center for Eosinophilic Disorders
CCHMC:	Cincinnati Children's Hospital Medical Center
DZ:	Dizygotic
EoE:	Eosinophilic esophagitis
MZ:	Monozygotic
RRR:	Recurrence risk ratio

METHODS

To quantify the risk of EoE due to genes and the 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 Institutional Review Board approval and review by the University of Cincinnati Institutional Review Board. Participants or their parent/guardians provided written consent. Children older than 11 years provided written assent.

The CCED database was used for the period August 1, 2008, to April 30, 2013, to identify patients and collect basic demographic characteristics, 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 a previsit questionnaire with subsequent physician confirmation, available in CCHMC's electronic medical record. Family medical conditions included EoE and other eosinophilic gastrointestinal diseases, 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 (67%) 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 eosinophilic gastrointestinal disease. EoE Twins are from the continental United States (n = 57), Alaska (n = 2), and Australia (n = 4). Information for twins younger than 18 years was provided by parent report.

Inclusion and exclusion criteria

Eligible participants/parents were asked for reported diagnosis (EoE, other gastrointestinal conditions, or unaffected). For all participants who reported EoE, the esophagogastroduodenoscopy 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 (M.H.C.) for the area (0.3 mm^2) of greatest intraepithelial eosinophil density. Peak counts were generated (100% of Nuclear-Family; 96% of Twins) to confirm 15 or more eosinophils per hpf at 400× magnification. Slides were requested from an endoscopy performed while the participant was receiving proton pump inhibitor therapy but had not received therapy specifically for EoE, such as steroids and/or diet elimination, as recommended in the EoE consensus guidelines.[7] Proton pump inhibitor administration before a positive endoscopy was confirmed in 52% of Nuclear-Family probands for whom data were available (55%). Affected Twins diagnostic dates ranged from 2001 to 2012, with 93% diagnosed before the publication of the current guidelines recommending proton pump inhibitor screening before 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 demographic characteristics (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 or younger; the prepIT L2P manual DNA purification protocol was used.

Zygosity

Three tools determined the zygosity of same-sex twins as monozygotic (MZ) or dizygotic (DZ): (1) genotyping, (2) the pea pod questionnaire,[32] and (3) parent report. To genetically determine zygosity, we estimated the proportion of identity-by-descent sharing between each pair of genotyped individuals and compared it with the proportion expected on the basis of genealogical information.[33] The percentage of identical markers was determined from 94,544 high-quality, polymorphic markers, among 196,524 variants genotyped by Immunochip[34] (Illumina, San Diego, Calif). MZ pairs have identical markers at more than 99% of loci, with observed identity-by-descent sharing of 0.99 to 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, the pea pod questionnaire determined zygosity. The pea pod questionnaire is a validated survey designed to determine how alike twins are on the basis of who can tell them apart,[32] with 96% accuracy relative to genotyping.[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 the CCHMC.[36]

Environmental screening

Because EoE often has an early onset, we focused on perinatal exposures, such as prenatal vitamins, gestational age, breast-feeding, 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 eosinophilic gastrointestinal disease database for the Nuclear-Family cohort and by telephone interview for the Twins cohort 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 2-tailed with significance at $P \leq .05$, unless otherwise specified; exact values at $P \geq .001$ or $P < .001$ were confirmed by permutation test for zero cells.

Demographic characteristics were described using mean ± 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 nonparametric Wilcoxon rank sum test, parametric t tests, Fisher exact test, or the χ^2 test, as appropriate.

Recurrence risk ratios and concordance estimates

Recurrence risk ratios (RRRs) were calculated as (number affected/total)/prevalence, with the point estimate for prevalence set at 5.5 per 10,000.[1-3] Given the male preponderance of EoE, sex-adjusted frequencies and sex-stratified RRRs were calculated; prevalence per 10,000 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 (χ_1^2). Probandwise concordance, which provides an estimate for agreement of disease state between twins while accounting for ascertainment, was calculated as $2C/(2C + D)$,[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 could not be separated in nuclear families, we denoted this heritability as combined gene-environment (h_{gc}^2). Details are specified in this article's Online Repository at www.jacionline.org.

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 because of small sample size. Nonparametric Wilcoxon rank sum test, parametric t tests, or the chi-square test 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 (67%). After excluding grandparents (n = 2391) and twin families (n = 31), the Nuclear-Family cohort comprised 914 probands and 2192 first-degree relatives (n = 3106) (Fig 1). 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, the 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 versus DZ pairs ($P = .96$). 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 ($P < .001$) and older ($P = .006$; 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 to 13.2 years, with interquartile ranges of approximately 7.7 to 19.1 years. Interestingly, both cohorts had 73% to 74% males, 87% to 94% whites, and 94% non-Hispanics.

Frequency, recurrence, and concordance of EoE

To characterize familial clustering of EoE, we first calculated the 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%; $P = .004$) and brothers (3.5%; $P < .04$) had EoE at significantly higher rates than did mothers (0.6%) and sisters (1.3%), respectively. The EoE frequency in both MZ (41.0%) and DZ (22.0%) twins was significantly higher than in siblings (Fig 2). Surprisingly, the EoE frequency in DZ twins was higher than in nontwin siblings from the Nuclear-Family cohort ($P < .001$, Fig 2).

Compared with the general population, the risk of EoE for first-degree relatives from the Nuclear-Family cohort (n = 2192) was increased; the RRR (RRR = λ_R) was highest in brothers (64.0; $P = .04$) and fathers (42.9; $P = .004$) compared with sisters (24.0) and mothers (9.9), respectively. Males had higher RRRs

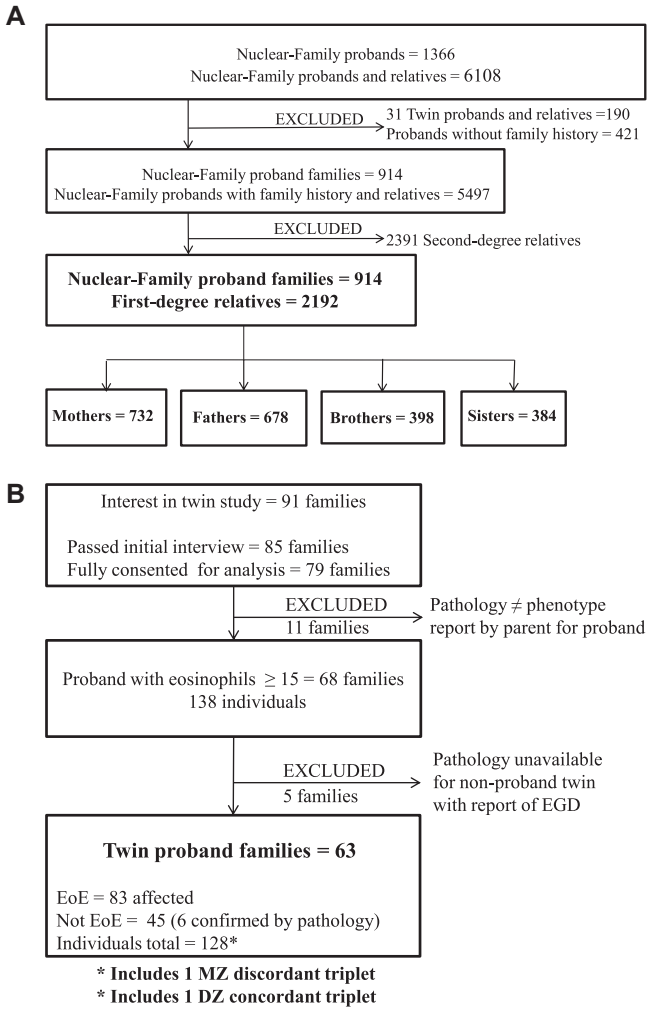

FIG 1. Recruitment algorithms and case identification for Nuclear-Family and Twin cohorts. **A,** Nuclear-Family cohort. **B,** Twin cohort. *A,* Nuclear-Family cohort from the CCED. *B,* EoE Twins International Registry cohort; *EGD,* esophagogastroduodenoscopy; *not EoE,* unaffected by EoE.

than did females (50.7 vs 14.7; $P < .001$) (Table II). Sibling RRR compared with parent RRR (44.2 vs 25.8; $P = .09$; 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 ($_{adj}\lambda_R = 28.2$).

Probandwise concordance in MZ co-twins was 57.9% ± 9.5% compared with 36.4% ± 9.3% in DZ twins. Although these concordances were not significantly different from each other ($P = .11$), the higher rates of EoE in MZ compared with 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 (Fig 3). Traditional Mendelian inheritance includes dominant, recessive, and X-linked patterns. In dominant inheritance, transmission between an affected parent and a child is approximately 50%; however, in the Nuclear-Family cohort, 98% of the probands have unaffected parents. Autosomal-recessive inheritance often has children with unaffected parents, but approximately 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 the families had at least 1 additional EoE-affected sibling. Last, 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% ($P < .001$; SE = 0.027) of the total phenotypic variance, suggesting a strong effect from genetics. Because all twin pairs share common environmental exposures, but MZ and DZ twins vary in genetic sharing, their phenotypic variance can further be partitioned into additive genetic (A), common environment (C) and unique environment/error (E) using the ACE model. Therefore, parallel analyses in twins estimated the combined AE "heritability" (h_{gc}^2) at 99.5% ($P < .001$). However, the model that separates genetic heritability and common environment (ACE, goodness-of-fit $P = .56$) fit the data better than did either the model with genetics (AE, goodness-of-fit $P < .001$) or the model with common environment (CE, goodness-of-fit $P = .006$) (Table III), suggesting that the EoE risk resulted from both genetic and shared environmental factors. Importantly, the heritability (estimate 14.5% ± 4%; $P < .001$; Fig 4, *A*) 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%; $P < .001$; Fig 4, *A*). Thus, heritability estimates are markedly inflated when common environment is not accounted for (Fig 4, *B*).

Evidence for shared environmental effects

Given increased EoE rates in DZ twins compared to in nontwin siblings, we tested environmental factors that may be shared between twin pairs but not necessarily between siblings. Although the sample size was limited, greater differences in birth weight were associated with disease discordance in twin pairs ($P = .01$; n = 35; Table IV). Birth season was significantly different in concordant and discordant twin pairs ($P = .03$; n = 63); specifically, birth in fall was associated with EoE discordance ($P = .02$; n = 63). Food allergies ($P < .001$; n = 97) were associated with EoE, and penicillin allergies ($P = .17$; n = 66) and breast-feeding ($P = .15$; n = 59) may influence the risk for EoE.

DISCUSSION

Previous studies reported familial clustering of EoE,[8,38-43] suggesting that clustering is attributable to genetics. Indeed, our large Nuclear-Family cohort 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

TABLE I. Demographic characteristics of EoE Nuclear-Family and Twin cohorts

Characteristic	Nuclear-Family	Twin		
		All	MZ	DZ
All families (n)	914	63	28	35
Male sex (%)	74.0	73.4	92.9*	58.3*
Race (%)				
White	86.7	93.7	100.0	88.6
Black	3.9	0	0	0
Asian	0.7	0	0	0
AI/AN	0.3	0	0	0
Other	8.4	6.4	0	11.4
Ethnicity (%)				
Non-Hispanic	94.2	93.7	96.4	91.4
Hispanic	1.9	3.2	3.6	2.9
Missing	3.9	3.2	0	5.7
Age (y), median (IQR), range	12.3 (7.7-17.2), 1-64	13.2 (8.1-19.1), 3.0-51.8	15.8† (8.3-32.0), 6.2-51.8	10.2† (7.9-16.7), 3.0-34.9

AI/AN, American Indian or Alaska Native; *IQR*, interquartile range.
*MZ > DZ male sex ($P < .001$).
†MZ > DZ age ($P = .006$). All others: not significantly different by the chi-square test, Fisher exact test, or Wilcoxon nonparametric test.

TABLE II. Frequency and recurrence risk ratios (λ_R) in EoE Nuclear-Family cohort first-degree relatives

First-degree relative	Frequency (%)	*P* value	Sex-adjusted frequency (%)	RRR (frequency/ prevalence)	Sex-stratified RRR
All	1.8			32.5	—
Males	2.8*			50.7*	34.3
Females	0.8	<.001	2.3	14.7	28.2
Parents	1.4			25.8	—
Fathers	2.4*			42.9*	29.0
Mothers	0.6	.004	1.9	9.9	19.1
Siblings	2.4			44.2	—
Brothers	3.5*			64.0*	43.2
Sisters	1.3	.04	2.9	24.0	45.5

Prevalence at 5.5/10,000; sex-stratified prevalence per 10,000 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.
*Unadjusted $P < .05$ by $\chi^2_{df = 1}$.

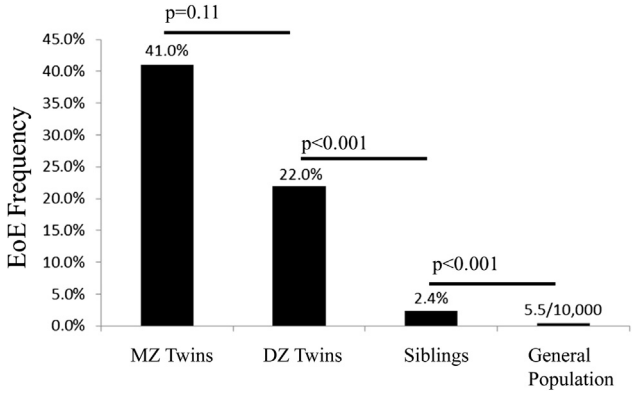

FIG 2. Rates of EoE in Twin cohort and Nuclear-Family cohort sibling nonprobands. Frequency of EoE in DZ nonproband co-twins (n = 36) and nonproband Nuclear-Family siblings of proband (n = 782) compared with population prevalence by χ^2df = 1.

(proportion of variation explained by genes) estimate. However, 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 the EoE risk, whereas fall birth season and breast-feeding may reduce the risk, supporting further exploration of early life factors. Thus, we propose that disease susceptibility in genetically predisposed families may be potentiated by early life environment. Notably, colonization by immune-shaping commensal microbiota, in the gut and also in the esophagus,[44-47] could be a key determinant of environmental risk.

First-degree relatives of probands have a higher rate of EoE than does the general population

In the 1.9% of families in the Nuclear-Family cohort that had at least 1 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.[8] Compared with other allergic diseases, such as asthma with sibling RRR between 1.25 and 2.25,[48] 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 1 additional child who also has EoE (1.9%; Fig 3) 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,[38] and, indeed, Nuclear-Family heritability was estimated at 72%. A strong genetic basis for EoE is further supported by candidate and genomewide association studies that identified risk variants,[9,11-13] as well as

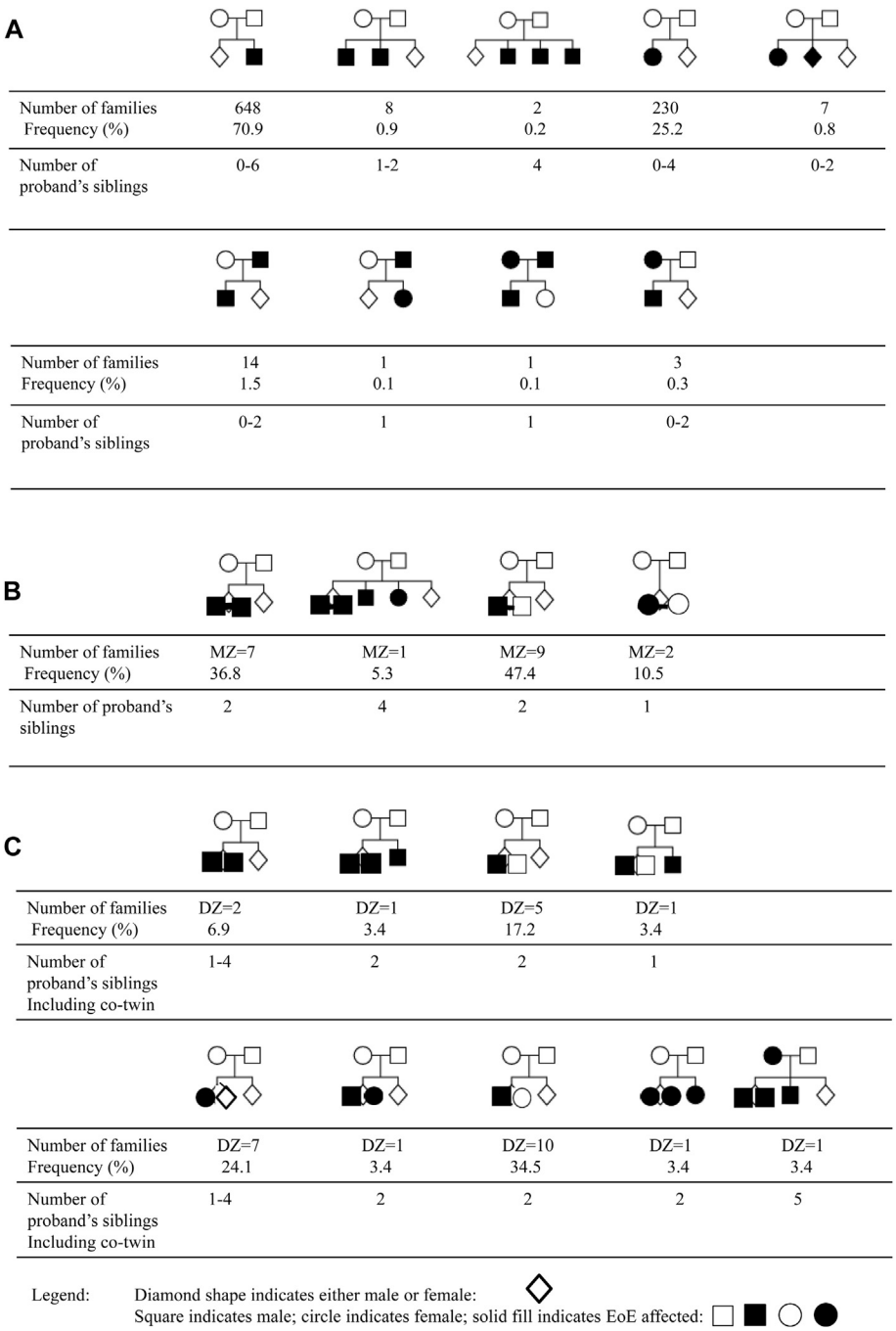

FIG 3. Summary pedigrees support a complex mode of EoE inheritance. **A,** Nuclear-Family cohort. **B,** Twin cohort (MZ). **C,** Twin cohort (DZ). *Diamond shape* indicates male and/or female, as it represents both brothers and sisters whose number range by "Number of proband's siblings." Frequency (%) is the percentage of families with that summary pedigree as a percentage of all families in panels *A*, *B*, and *C*. In the large Nuclear-Family cohort, families with unaffected parents and at least 1 additional brother or sister with EoE comprise 1.9%.

EoE-specific gene expression profiles.[10] However, estimating heritability from nuclear families has limited interpretation because genes and family environment cannot be distinguished.[49,50] 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 those of the common family environment.[51,52] Indeed, the heritability estimate from the reduced AE model (combined gene-environment heritability, which ignores the common family environment) was inflated (99.5%). This high

TABLE III. Nested ACE twin models to estimate heritability

Model	Twin pair intraclass correlation		Parameter estimates			Model fit	
	MZ	DZ	ag^2	c^2	e^2	$\chi^2_{(df)}$	*P* value
ACE	0.955	0.883	0.145	0.810	0.045	2.04 (3)	.56
CE	0.940	0.940	—	0.94	0.060	14.64 (4)	.006
AE	0.995	0.498	0.995	—	0.005	489.92 (4)	<.001

Nonsignificant *P* value for χ^2 indicates superior fit of the model to the data.
A (ag), Additive genetic; *C (c)*, common environmental exposures; *E (e)*, error due to unique environmental exposures.

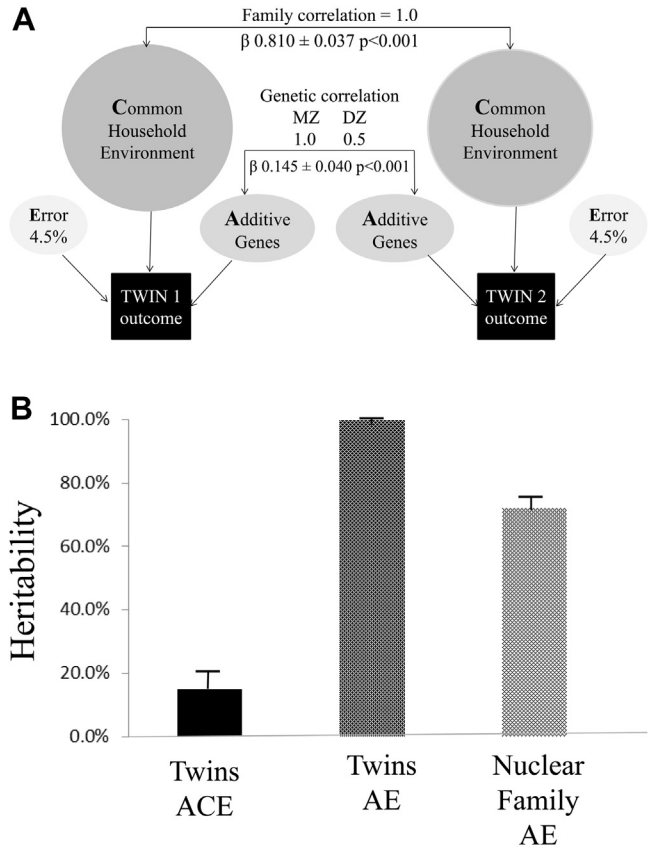

FIG 4. A, Twin cohort ACE model more accurately estimates heritability by separating common environment. **B,** Twin cohort ACE heritability model estimates compared with Twin cohort AE and Nuclear-Family AE cohort estimates. Fig 4, *A*, "ACE" latent class path analysis estimates (point prevalence estimate 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. Fig 4, *B*, Twin cohort ACE path analysis *(black)* separates common family environment, estimating heritability at 14.5% ± 4% (*P* < .001) with superior model fit (*P* = .56). As expected, using the same data and model but excluding the common family environment *(dark gray)* inflates heritability to 99.5%. Similarly, Nuclear-Family cohort *(light gray)* inflates heritability estimate to 72% ± 2.7% (*P* < .001; liability threshold model). *A*, Additive genetic variance (heritability); *C*, common, shared household, environmental variance; *E*, unique environment "error" variance.

value is not unexpected because twin models often produce inflated estimates[53] because of 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 with nontwin 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,[54-57] and "phantom" heritability is speculated to be the result of genetic interactions.[51] Our results show that the amount of variation attributed to genetic factors is overestimated because of failure to account for the common family environment.

Early life exposures likely contribute

Our results suggest that early life exposures likely contribute to EoE risk. The high concordance of EoE for DZ twins compared with nontwin siblings is unexpected because both nontwin 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 have an important role in EoE pathogenesis.[58-61] Preliminary family environmental data suggest that factors in early life, such as birth season, breastfeeding, and penicillin allergy 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.[18] Previous studies and our data substantiate the importance of early life exposures, such as antibiotics,[62-64] specifically penicillins and cephalosporins[65] that alter gut colonization, likely reflecting the role of the metagenome and early microbiota and helminth colonization in priming the developing immune system.[44-47] Parent-/self-report of penicillin-like allergies in twins differentiates concordant and discordant pairs. Furthermore, young children ingest food, water, juice, airborne particles, soil, and dust exposure doses many times higher than do adults,[66] presenting an opportunity for the identification of novel environmental risk factors that alter expression at an early age. An environmental effect on EoE risk is plausible given the dynamic nature of the EoE transcriptome, which varies with allergen exposure (eg, diet).[10,31] Our breastfeeding data suggest a protective effect against EoE, consistent with current recommendations.[67] Although birth weight differences between twins and birth season may affect outcomes, they are less modifiable. These data should be interpreted with caution given the 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 the common family environment. Evidence-based risk assessment data show that, overall, the sibling risk is modest (2.4%). 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 the 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, it is hoped that future studies will facilitate the translation of these findings to actionable recommendations.

TABLE IV. Preliminary screen of environmental and comorbid risk factors in the Twin cohort

A. Twin pair

Exposures for pairs (maximum n = 63)	n	Discordant frequency, % or mean ± SD	Concordant frequency, % or mean ± SD	*P* value	OR	CI_{95}
Current age (y)	63	16.3 ± 11.3	16.0 ± 11.8	.96	1.0	—
Gestational age (wk)	43	35.0 ± 3.4	35.0 ± 2.2	.58	—	—
Preterm birth (≥33.5 wk)	43	75.8	80.0	1.00	1.3	0.2-7.3
Term birth (≥35 wk)	43	50.0	69.7	.25	0.4	0.1-1.8
Twin birth weight difference (g)	35	335.7 ± 273.0	145.6 ± 133.7	.01	—	—
Birth season adjusted for hemisphere	63			.03	—	—
Fall		43.2	10.5			
Winter		13.6	31.6			
Spring		18.2	10.5			
Summer		25.0	47.4			
Birth season fall	63	43.2	10.5	.02	0.2	0.03-0.8
Fertility treatments	47	45.7	33.3	.52	0.6	0.2-2.3
Fertility treatment (by type)	20	Sparse data		.43	—	—
Chorion/Amnion number	32	Sparse data		.56	—	—
Prenatal vitamins	44	93.9	100	.99	—	—
Birth order (twins only)	45	47.1	54.6	.67	1.4	0.3-5.3
Penicillin allergy in family	44	21.2	36.4	.42	2.1	0.5-9.4

B. Individual twin

Individual twin exposures (maximum n = 128)	n	Not EoE	EoE	*P* value	OR	CI_{95}
Breast-feeding	59	90.0	65.3	.15	0.2	0.02-1.8
Birth order (second, twins only)	91	47.1	50.1	.72	1.2	0.5-2.7
Birth weight (g)	80	2400 ± 663	2358 ± 532	.77	—	—
Birth length (in)	38	19.2 ± 1.4	18.6 ± 1.1	.24	—	—
Allergies, environmental	97	64.7	76.2	.23	1.7	0.7-4.3
Allergies, spring	69	90.9	83.0	.48	0.49	0.09-2.5
Allergies, summer	13	66.7	80.0	1.00	2.0	0.1-34.8
Allergies, fall	68	86.3	87.0	1.00	1.1	0.2-4.7
Allergies, winter	66	59.1	61.4	.86	1.1	0.4-3.1
Allergies, year round	66	61.9	64.4	.84	1.1	0.4-3.3
Food allergies	97	23.5	81.0	<.001	13.8	5.0-38.0
Penicillin allergy	66	0.0*	100.0	.17	—	—

CI_{95}, 95th percentile for CI; *OR*, odds ratio.
*Confirmed by using the 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 exact test was used for discrete variables; Student *t* test for continuous variables.

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HERITABILITY ANALYSES

In the Nuclear-Family cohort, heritability was modeled with liability thresholds and variance components modeling using sequential oligogenic linkage analysis routines (SOLAR; Texas Biomedical Research Institute, San Antonio, Tex).[E1] Briefly, this approach decomposes the trait's phenotypic variance into additive genetic variance and residual effects operationalized as follows:

$$\Omega = 2\mathbf{\Phi}\sigma_{\mathrm{ag}}^2 + \mathbf{I}\sigma_{\varepsilon}^2$$

Ω is the covariance between a pair of relatives and captures phenotypic variance (V), $\mathbf{\Phi}$ is the kinship coefficient matrix, $\sigma_{\mathrm{ag}}{}^2$ is the additive genetic variance, $\mathbf{I}$ is the identity matrix, and $\sigma_{\varepsilon}{}^2$ is the residual variance due to stochastic error ("noise") and unique environment. In this model, only $\sigma_{\mathrm{ag}}{}^2$ and $\sigma_{\varepsilon}{}^2$ are estimated with the matrices defined *a priori*. To account for ascertainment, the sample mean was set to population prevalence (5.5/10,000).[E2-E4] To assess significance of the genetic variance component, twice the difference between the log-likelihoods of this model and one without the genetic component was computed and compared with a χ_1^2 distribution. *Heritability* was defined as $\sigma_{\mathrm{ag}}{}^2/(\sigma_{\mathrm{ag}}{}^2 + \sigma_{\varepsilon}{}^2)$. However, given the nuclear family design, this heritability estimate is denoted as $h_{\mathrm{gc}}{}^2$ (combined gene-environment heritability) to account for the fact that the common family environment (C) cannot be separated from additive genetic effects.

To appropriately account for twin relationships, structural equations modeling was applied[E5-E8] using Mplus (Mplus: Muthén & Muthén, Los Angeles, Calif). Briefly, these models examine the covariance within and between twins. Importantly, jointly estimating effects in MZ and DZ twins, variation can be portioned into genetic and environmental components (AE model; additive genetic [A] and unique environment/error [E]). However, because all twin pairs share common environmental exposures, variation can further be partitioned into additive genetic (A), common environment (C), and unique environment/error (E) using the ACE model. Using the terminology of variance components analysis, the ACE model can be operationalized as follows:

$$\Omega = 2\mathbf{\Phi}\sigma_{\mathrm{ag}}^2 + \mathbf{C}\sigma_{\mathrm{C}}^2 + \mathbf{I}\sigma_{\varepsilon}^2$$

where $\mathbf{C}$ is a matrix used to derive the variance explained by the common family environment ($\sigma_{\mathrm{C}}{}^2$). Model constraints included intrafamily environmental correlation (C) at 1.0 for both types of twins and genetic component (A) correlation at 1.0 for MZ twins and 0.50 for DZ twins. Ascertainment was corrected by incorporating the point estimate of prevalence of 5.5 per 10,000 in the population.[E1-E3] As with variance components modeling, significance of effects was determined by comparing the log-likelihoods of nested models for all 3 combinations of ACE, AE, and CE models to determine the best-fitting, data-driven model. Nonsignificant P values indicate better model fit to the data. Heritability can be measured from both the AE and ACE models. However, heritability from the AE model does not separate additive genetic effects from shared family environment effects; it is designated as $h_{\mathrm{gc}}{}^2$. Heritability from the ACE model uniquely separates additive genetic effects and thus is designated $h_{\mathrm{ag}}{}^2$.

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