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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This study was supported in part by the: Frank C. Woodside, Dinsmore & Shohl Fellowship through Cincinnati Children's Division of Biostatistics and Epidemiology; University of Cincinnati Research Council; Campaign Urging Research for Eosinophilic Diseases (CURED); Food Allergy Research and Education (FARE); Buckeye Foundation; National Institutes of Health (NIH) grants: T32-ES10957 Molecular Epidemiology in Children's Environmental Health Fellowship 2011-2013; NIEHS P30-ES006096 Center for Environmental Genetics New Investigator Scholar and PI Mentee/Mentor; NIH 8 UL1-TR000077-04 Center for Clinical and Translational Science and Training, CTSA, NCATS Just in Time; CCTST REDCap UL1-RR026314-01 NCRR/NIH; 1R25GM093044-01 UAB Section on Statistical Genetics; NIH-1K24DK100303 (GTF). This work was completed in partial fulfillment of the Doctor of Philosophy degree in Epidemiology in the Department of Environmental Health, Division of Epidemiology and Biostatistics, University of Cincinnati College of Medicine.

Disclosure of potential conflict of interest: E. S. Alexander has received research support from the National Institutes of Health (NIH), the University of Cincinnati Research Council, and the 2014 Frank C. Woodside, Dinsmore & Shohl Fellowship/Cincinnati Children's Hospital Division of Biostatistics and Epidemiology; has received NIH travel support; and has received payment for lectures from Xavier University. L. J. Martin has received NIH research support. M. H. Collins is on the Medical Advisory Panel for American Partnership for Eosinophilic Disorders (APFED); is on the executive committees for The International Gastrointestinal Eosinophils Researchers (TIGERS) and the Registry for Eosinophilic GastroIntestinal Disorders (REGID); and has consultant arrangements with Meritage Pharma, Regeneron, Receptos, and Novartis. H. Sucharew has received NIH research support. J. P. Abonia has received NIH research support and funds from FARE, the Buckeye Foundation, and the CURED Foundation. H. Foote's work was funded by NIH research support. E. S. Dellon has consultant arrangements with Aptalis, Novartis, Receptos, and Regeneron; has provided expert testimony for Child-Reed; and has received research support from Meritage and AstraZeneca. J. G. Demain is employed by the Allergy, Asthma & Immunology Center of Alaska and has received research support from the American Academy of Allergy, Asthma & Immunology. G. T. Furuta has received NIH research support, has consultant arrangements with Genentech, has patent for an esophageal string test, and is cofounder of EnteroTrack. P. E. Putnam has received payment for lectures from Abbott Nutrition and Nutricia. M. E. Rothenberg has received NIH research support; has research funds from the CURED Foundation, the Buckeye Foundation, and FARE; has consultant arrangements with Immune Pharmaceuticals, Pluristem Pharmaceuticals, Receptos, Inc, and Novartis; is an inventor for patents owned and submitted by Cincinnati Children's Hospital Medical Center; and has stock/stock options in Immune Pharmaceuticals and Receptos. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication May 16, 2014; revised June 27, 2014; accepted for publication July 3, 2014.

Available online September 22, 2014.

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0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2014.07.021

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;134:1084-92.)

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