High prevalence of eosinophilic esophagitis in patients with inherited connective tissue disorders

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Background: Eosinophilic esophagitis (EoE) is an emerging chronic inflammatory disease mediated by immune hypersensitization to multiple foods and strongly associated with atopy and esophageal remodeling.
Objective: We provide clinical and molecular evidence indicating a high prevalence of EoE in patients with inherited connective tissue disorders (CTDs).
Methods: We examined the rate of EoE among patients with CTDs and subsequently analyzed esophageal mRNA transcript profiles in patients with EoE with or without CTD features.
Results: We report a cohort of 42 patients with EoE with a CTD-like syndrome, representing 0.8% of patients with CTDs and 1.3% of patients with EoE within our hospital-wide electronic medical record database and our EoE research registry, respectively. An 8-fold risk of EoE in patients with CTDs (relative risk, 8.1; 95% confidence limit, 5.1-12.9; $\chi^2_1 = 112.0$; $P < 10^{-3}$) was present compared with the general population. Esophageal transcript profiling identified a distinct subset of genes, including *COL8A2*, in patients with EoE and CTDs.
Conclusion: There is a remarkable association of EoE with CTDs and evidence for a differential expression of genes involved in connective tissue repair in this cohort. Thus, we propose stratification of patients with EoE and CTDs into a subset referred to as EoE-CTD. (J Allergy Clin Immunol 2013;132:378-86.)

Key words: *Eosinophilic esophagitis, eosinophilic gastrointestinal disease, eosinophil, connective tissue disorders, Ehlers-Danlos syndrome, Marfan syndrome, hypermobility syndrome*

Eosinophilic esophagitis (EoE) is an emerging worldwide immune-mediated disease characterized by intense eosinophil infiltration of the esophageal mucosal epithelium that is refractory to acid-suppressive therapy and often associated with significant tissue remodeling.[1] First described in the late 1970s, the incidence and prevalence of EoE has been on the increase. It is now a global health disease reported in every continent except Africa and has been shown to affect approximately 1:1000 subjects.[2,3] EoE typically occurs as an isolated disease entity, although it is often associated with concurrent allergic disease, including asthma, eczema, food-induced anaphylaxis, and polysensitization to antigens, especially food allergens. An allergic cause for EoE is supported by the reversibility of the disease after dietary avoidance of specific foods,[4] the reoccurrence of the disease on reintroduction of the removed foods,[5-7] the induction of the disease in mice by exposure to allergens,[8] and genome-wide transcriptome analysis of esophageal tissue having implicated an interplay of innate and adaptive T_H2 immunity.[9] The disease has a strong hereditary component with a large sibling risk ratio ($\lambda_s \sim 80$),[9] and early genetic analyses have identified susceptibility loci in regions that contain candidate genes that are expressed in epithelial cells and strongly implicated in regulating antigen recognition (thymic stromal lymphopoietin *[TSLP]*) and inflammatory cell recruitment and activation (eotaxin-3 *[CCL26]*).[10-12]

Among the many immune-modulating molecules implicated in EoE disease pathogenesis, recent attention has focused on TGF-β1, the levels of which are increased in the esophagus in patients with EoE and localized to eosinophils within the inflamed and fibrotic esophageal lamina propria, along with deeper-lying

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Abbreviations used
BMI: Body mass index
CCHMC: Cincinnati Children's Hospital Medical Center
CTD: Connective tissue disorder
EDS: Ehlers-Danlos syndrome
EGID: Eosinophilic gastrointestinal disorder
EMR: Electronic medical record
EoE: Eosinophilic esophagitis
FBN1: Fibrillin-1 gene
GERD: Gastroesophageal reflux disease
i2b2: Informatics for Integrating Biology & the Bedside
JHS: Joint hypermobility syndrome
LDS: Loeys-Dietz syndrome
MFS: Marfan syndrome
PPI: Proton pump inhibitor

mast cells found within the esophageal smooth muscle layer.[13-15] TGF-β1 not only promotes smooth muscle contractility and fibrosis, processes that might be involved in eliciting the esophageal dysfunction seen in patients with EoE, but also is a key immunomodulating cytokine that is expressed by and essential for the function of regulatory T cells. Notably, an increased level or imbalance of regulatory T cells occurs in patients with EoE and in animal models of EoE.[16,17]

A series of Mendelian-inherited connective tissue disorders (CTDs) are caused by genetic variants in TGF-β binding proteins (eg, Marfan syndrome [MFS]) and TGF-β receptors (Loeys-Dietz syndrome [LDS]), and excess TGF-β1 levels and pathway signaling have been associated with these 2 disorders.[18,19] Although the Ehlers-Danlos syndromes (EDSs) have not been directly associated with excess TGF-β1 levels, direct protein and regulatory interactions between TGF-β1 and a mutated *COL5A1* collagen protein have been reported.[20-22] It is notable that these disorders are often associated with gastrointestinal symptoms,[23,24] especially dysphagia,[25] which also represents a chief symptom of EoE in adults. Although eosinophilia has not been previously associated with CTDs outside of collagen vascular diseases such as scleroderma, dermatomyositis, and polymyositis,[26,27] we recently began to encounter patients with EoE who had coexisting CTDs without the features of autoinflammatory collagen vascular disease. Herein, we describe a new syndrome involving the coexistence of EoE with CTD (EoE-CTD). Although some patients with EoE-CTD have known causative mutations in CTD genes, they did not manifest the full phenotype of CTDs but rather had an enrichment of Marfanoid features and extensive hypermobility.

METHODS

Patients

The patients used in these analyses came from 2 primary data sources: an Informatics for Integrating Biology & the Bedside (i2b2) data warehouse and our eosinophilic gastrointestinal disorders (EGIDs) research database. The i2b2 warehouse represents a deidentified database of all patients seen at Cincinnati Children's Hospital Medical Center (CCHMC) whose data are derived from our local implementation of the EPIC electronic medical records (EMRs) containing patients' records from March 2007 through December 2012.[28,29] Our EGID database contains patients with EoE and control subjects. All participants registered in the EGID database have undergone a formal informed consent process approved by the CCHMC Institutional Review Board, with data collection beginning in approximately June 2000 and continuing through December 2012. Data collected include demographics, clinical testing, and past medical, surgical, and family histories, along with samples (blood DNA and esophageal biopsy mRNA). However, data and samples available for participants varied, and thus the specific subjects entering each type of analysis are described below. Importantly, a simple majority of the patients with EoE at our medical center are included in the EGID database (58%). Patient phenotypes have been described previously.[9,30] In brief, patient phenotypes for mRNA analyses are as follows: *control subjects* (n = 12), patients with no EoE diagnosis with normal esophageal histology; *patients with active EoE* (n = 12), peak esophageal eosinophil count of 15 or more per high-power field; *patients with EoE-CTD* (n = 6), peak esophageal eosinophil count of 15 or more per high-power field with a coexisting CTD. The diagnosis of EoE was confirmed based on proton pump inhibitor (PPI) administration before a positive endoscopic result in 47% of patients with EoE-CTD for the mRNA analyses. Control subjects had not been given a diagnosis of EoE or other related gastrointestinal conditions and had normal esophageal histology. For this study, slides of the biopsy specimens obtained at endoscopy that yielded tissue for mRNA extraction (see below) from the patients with EoE-CTD were reviewed by a single pathologist (M.H.C.) and analyzed for peak eosinophil counts and histopathologic features associated with EoE. For analysis of height, weight, body mass index (BMI), and age, the EoE-CTD group (n = 42; male, n = 29; female, n = 13) was identified based on clinical evaluations by physicians in the fields of allergy, gastroenterology, and genetics. Of these patients with EoE-CTD, 10 (24%) had evidence of eosinophilic gastrointestinal disease outside of the esophagus (stomach, n = 7; duodenum, n = 3; and colon, n = 1). This extraesophageal disease is defined as an eosinophil count in excess of that reported in Debrosse et al,[31] along with evidence of architectural destruction (as evaluated by M.H.C.) while also fulfilling the criteria for eosinophilic gastritis, as suggested by Lwin et al.[32]

For the mRNA analysis cohort, subjects had no evidence of extraesophageal eosinophilic gastrointestinal disease, and evidence of active EoE was determined by rereview of available slides or from data previously collected within the EGID database. For comparison with patients with EoE-CTD, 42 control subjects and 42 patients with EoE without CTDs were randomly selected from the EGID database, excluding the 42 patients with known EoE-CTD. These control subjects and patients with EoE without CTDs in the EGID database were assigned a random number derived from a globally unique identifier, sorted by random number value, and selected to match the same distribution of male and female patients seen for the EoE-CTD patient population.

Thirty-six percent (15/42) of patients with EoE-CTD responded to a variety of dietary treatments based on review of clinical records. No patients in this report underwent esophageal manometry, and a small number of patients (5/42) underwent barium swallow evaluation, none of whom had structural abnormalities. Videos E1 to E4 demonstrating evidence of the joint hypermobility seen in these patients with EoE-CTD can be found in this article's Online Repository at www.jacionline.org. Releases for the use of these videos were obtained from the parents or a single adult subject. This study was approved by the Institutional Review Board of CCHMC.

Comparison of rates of EoE, CTD, and EoE-CTD

To determine the total number of patients and the numbers of patients with EoE, CTD, and EoE-CTD, we used the i2b2 workbench.[28,29] The following specific diagnostic codes were used to identify patients with CTDs: Marfan and Marfanoid-related syndromes, 759.82, 759.82F, 759.82Q, and 759.82R; Ehlers-Danlos and related syndromes, 756.83, 756.86.CQ, 756.86CT, 756.83CU, 756.83.DL, 756.83.DM, 756.83.DN, 756.83EL, and 756.83X; and Loeys-Dietz syndrome, 759.89ALK. The specific diagnostic codes 530.13 and 530.19AQ were used to identify patients with EoE. Using the numbers of patients with EoE, CTDs, and EoE-CTD, we then compared the proportions of patients with and without EoE and CTDs by using 2 × 2 contingency tables to determine whether these 2 conditions occurred independently of each other. Gastroesophageal reflux disease (GERD) searches and diagnostic codes include 530.11J, 530.11AE, 530.11B, 530.11, 530.81BQ, 530.81AP, 530.81T, 530.81BG, 530.81AN, 530.81V, 530.81U, 530.81AV, 530.81Q, 530.81AK, 530.81N, 530.81B, 530.81AL, 530.81AX, 530,81AA, 530.81S, 530.81AZ, 530.81BY, 530.81AH, 530.81CC, 530.81R, 530.81CB,