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Original article

Comparison of gene expression profiles in eosinophilic esophagitis (EoE) between Japan and Western countries

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Abbreviations:
ALOX15, arachidonate-15 lipoxigenase; CAPN14, calpain 14; CCL26, C–C chemokine ligand 26; CCR3, C–C chemokine receptor type 3; CDH26, cadherin-like 26; CLC, Charcot-Leyden crystals; EG, eosinophilic gastritis; EGE, eosinophilic gastroenteritis; EGID, eosinophilic gastrointestinal disorders; EoE, eosinophilic esophagitis; FLG, filaggrin; GI, gastrointestinal; PMCH, pro-melanin-concentrating hormone; POSTN, periostin; TGF-β1, transforming growth factor-β1; TSLP, thymic stromal lymphopoietin

ABSTRACT

Background: The prevalence rate of eosinophilic esophagitis (EoE) between Japan and Western countries is quite different. Although multiple factors, including the genetic background, lifestyle and dietary habits, may account for the difference, the pathogenic mechanism of EoE has not been fully clarified in Japanese. To elucidate whether EoE's pathogenic mechanisms differ between those populations, we performed transcriptome analysis of esophageal biopsy specimens from Japanese EoE patients and compared the identified gene signatures with published microarray data for EoE patients in the US.
Methods: We prospectively enrolled adult Japanese EoE patients (n = 4) according to the 2011 consensus guidelines for diagnosis of EoE. Age-matched healthy volunteer subjects (n = 4) were also enrolled as controls. We assessed the gene expression profiles of esophageal biopsies using microarray technology and then compared the identified gene signatures with earlier data generated in the US.
Results: Of 42,545 transcripts represented on the microarray, 385 were differentially expressed between the EoE and control samples (≥2 fold change and adjusted p-value of <0.05). Our microarray data showed strong overlapping with the data from US patients with EoE. An EoE-specific-transcript signature is typically composed of IL-13-inducible and eosinophil-related genes, including eotaxin-3/C–C chemokine ligand 26 (CCL26).
Conclusions: This transcriptome study suggests that the pathogenetic mechanisms of EoE in Japan and Western countries are similar. Our findings may contribute to a better understanding of the pathogenesis of EoE and to more accurate diagnosis of this disease in Japanese individuals.

Introduction

Eosinophilic gastrointestinal disorders (EGID) are clinicopathologically characterized by massive eosinophilic infiltration within the gastrointestinal (GI) tract.[1] They are classified according to the site of infiltration as eosinophilic esophagitis (EoE), gastritis (EG), gastroenteritis (EGE), enteritis and colitis.[2] In Japan, the prevalence of EoE is reportedly quite low (estimated to be 0.01%),[3,4] and much less than that of EGE.[5] In contrast, the prevalence of EoE in Western countries, including the US and Europe, has been increasing in recent years, is now estimated to be 0.44–1%[6,7] and is much higher than that of EGE.[8] Notably, the prevalence of IgE-mediated food allergies (except for EGID) is increasing in Japan and shows a similar tendency to that in Western countries.[9,10] Thus, the reason for the approximate 50– to 100–fold difference in the prevalence rates of EoE between Japan and Western countries is unclear.

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Multiple factors, including the genetic background, lifestyle and dietary habits, may account for the difference in EoE between Japan and Western countries. To date, studies have suggested that EoE is more common in Caucasians than other racial groups.[11,12] Interestingly, EoE may have different clinical presentations and endoscopic findings between racially distinct populations. Moreover, dietary habits, especially a Mediterranean diet, are reportedly associated with development of allergic diseases.[13] The traditional diet of Japan is characterized by low fat content, even when consuming protein and dietary fiber, and it tends to prevent inflammatory bowel disease and colorectal cancer. In contrast, Western diets tend to promote those diseases. Since we have experienced EoE patients in spite of the Japanese genetic background and traditional Japanese dietary habits, the question arises as to whether EoE in Japan and Western countries is a different disease or not. A previous study of the pathogenic mechanism of EoE analyzed esophageal tissues by the transcriptome approach and found that eotaxin-3/C–C chemokine ligand 26 (CCL26) played a crucial role in inducing selective recruitment of eosinophils into the esophageal epithelium.[14]

In order to elucidate whether the pathogenic mechanism of EoE in Japan is similar to that in Western countries, we performed transcriptome analysis of esophageal biopsy specimens from Japanese EoE patients and compared the identified gene signatures with microarray data that have been published for EoE patients in the US.

Methods

We prospectively enrolled adult Japanese EoE patients (n = 4) who lived in the countryside and followed a traditional Japanese lifestyle and dietary habits. After granting informed consent, they underwent upper GI endoscopy due to their clinical symptoms. Diagnosis of EoE was defined as >15 eosinophils/HPF persisting in the distal esophagus even after proton pump inhibitor therapy, absence of treatment with oral or systemic steroids, in accordance with the 2011 consensus guidelines,[15] and exclusion of other possible causes of esophageal eosinophilia. Age-matched healthy volunteer subjects (n = 4) were also enrolled as controls. Control specimens were defined as having <1 eosinophils/HPF, with no history of treatment with oral or systemic steroids or EoE. All esophageal biopsies were obtained from the Department of Gastroenterology, Shimane University Hospital (Shimane, Japan). The study was performed according to a protocol approved by the institutional review board of National Center for Child Health and Development, Tokyo, Japan. Samples were placed in RNAlater® solution (QIAGEN, Valencia, CA, USA) at room temperature after biopsy and then stored at −80 °C until gene expression profiling.

Microarray analysis (Agilent Technologies, Santa Clara, CA, USA) was performed according to the manufacturer's instructions. Briefly, total RNA was extracted with an RNeasy Micro kit (Qiagen) and then evaluated with an Agilent Bioanalyzer and an RNA 6000 Nano kit (Agilent Technologies). The gene expression profiles were assessed using microarray technology with Agilent SurePrint G3 Human GE 8 x 60k. Data analysis was performed using GeneSpring software ver. 12.5 (Agilent Technologies). To normalize variation in the staining intensity between microarrays, the average difference for all genes on a given microarray was divided by the median of all measurements on that microarray, and genes in EoE patient specimens that showed a significant difference in signal intensity compared with the same genes in the control specimens ($P < 0.05$, t test) were considered to be up-regulated or down-regulated. Hierarchical clustering was performed using the gene expression data, contrasting the EoE and control specimens (see Fig. 1). The differentially expressed genes of EoE in Japan and the US were compared by systematic analysis using the NextBio search engine (http://www.nextbio.com/b/nextbio.nb)[16] (see Fig. 2).

Results

Characteristics of the subjects

The clinical and histopathological characteristics of all subjects are summarized in Table 1. All patients with EoE had a history of dysphagia, a common symptom observed among Japanese patients with EoE.[5] The most frequent endoscopic finding in patients with EoE was linear furrows, the diagnostic usefulness of which was recently reported.[17] Besides abnormal endoscopic findings, evidence of marked eosinophilic inflammation was observed in all patients with EoE.

Microarray analysis of differentially expressed genes in esophageal biopsies

Esophageal biopsy specimens from individual patients were subjected to whole-genome transcript expression profile analysis.

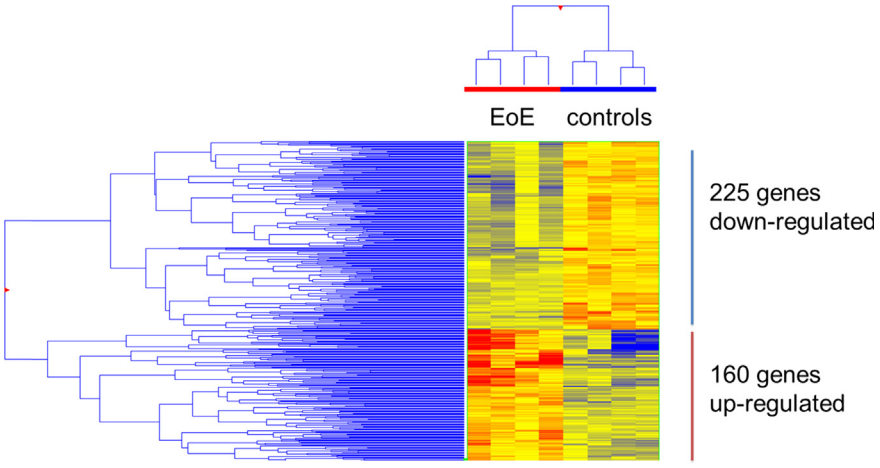

Fig. 1. Microarray analysis of differentially expressed genes in esophageal biopsies. The 385 genes differentially expressed ($P < 0.05$, fold difference ≥ 2) in the EoE group compared with control subjects are shown in a heatmap image. Up-regulated genes are represented in red and down-regulated genes in blue.