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ATG7 gene expression as a novel tissue biomarker in Eosinophilic Esophagitis

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Eosinophilic esophagitis (EoE) is a chronic allergic, immune-mediated disorder resulting in esophageal eosinophilia and dysfunction.¹ There is a strong need to identify additional biomarkers for confirmation of disease and response to treatment.^{2–5}

Autophagy (macroautophagy) is a cellular adaptive response to physiologic stressors such as starvation and inflammation that targets dysfunctional intracellular materials for degradation via autophagic vesicles.⁶ The formation of autophagic vesicles involves autophagy-related (ATG) gene products. ATG dysregulation has been implicated in immune disorders such as Crohn Disease⁷ and ATGs may serve as disease biomarkers.⁸ Thus, we hypothesized that ATG expression may be altered in esophageal biopsies from active EoE patients.

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Utilizing quantitative reverse transcription-polymerase chain reaction (qRT-PCR), we first evaluated expression of ATG3, ATG6, ATG7, ATG8 and ATG14 in a limited pediatric cohort. A full description of these methods is provided in the Methods section in this article's supplementary material. ATG6, ATG7 and ATG8 expression was higher in active EoE subjects (n=5) when compared to normal (n=5) and EoE remission subjects (n=5)(data not shown).

Further analysis was performed of a larger pediatric patient cohort (Table 1) including four patient groups: active EoE, EoE remission, Gastroesophageal Reflux Disease (GERD) and normal. ATG7 was upregulated in active EoE patients (n=27) compared to non-EoE (i.e. GERD and normal; n=26, p = 0.001) and EoE remission (n=24, p < 0.001)(Figure 1a) subjects. Logistic regression analysis of ATG7 level adjusted for age revealed an odds ratio of 12.1 (95% CI 3.0, 49.5) for active EoE. Moreover, the area under the receiver-operating characteristic (ROC) curve for the model's predicted value was 0.82 (Figure 1b). Therefore, ATG7 may serve as a valuable tissue biomarker distinguishing active EoE from remission and non-EoE states. Using 1.6 as an optimal cut point of the ATG7 expression level, patients can be classified as active EoE with 48.1% sensitivity and 88% specificity and positive and negative predictive values of 68% and 76%, respectively (Supplementary Table S1). While not a sensitive marker for active EoE, the remarkable specificity of ATG7 highlights its potential as a valuable diagnostic measure in conjunction with standard histopathological evaluation, particularly in cases with ambiguous eosinophil counts and or in children who are not on PPI at the time of endoscopy. Biomarker potential was further supported by a nearly significant (p=0.06) (data not shown) repeated measures analysis of ATG7 expression in a small cohort of EoE patients who transitioned from active EoE to EoE Remission or *vice versa* (n=18).

Linear regression analysis revealed only a modest positive correlation between ATG7 and eosinophilic infiltrate ($r^2 = 0.22$)(data not shown), suggesting that ATG7 elevation may represent a specific and independent marker of active EoE. To further validate this relationship, the non-EoE group was sub-stratified into normal (n=20) and PPI-treated GERD (n=6) patients; the latter defined by a history of reflux and evidence of histologic non-EoE inflammatory changes. ATG7 expression remained significantly elevated in patients with active EoE compared to normal (p = 0.001) and GERD (p= 0.017)(Figure 1a).

In summary, we have identified ATG7 as a potential tissue biomarker distinguishing active EoE from normal, EoE remission and GERD. Since subjects in this study were PPI-treated and exhibited minimal inflammation, further study is warranted in GERD subjects prior to PPI treatment to determine if ATG7 remains specific for active EoE. If this is the case, discovery of a specific tissue biomarker for EoE will provide clarity for ambiguous cases and may allow patients to avoid the recommended 8–12 week course of high dose PPI prior to esophagogastroduodenoscopy, resulting in earlier diagnosis and treatment.

Identification of autophagy related biomarkers in EoE may also be applicable to esophageal samples obtained from less invasive disease monitoring tools such as the esophageal string test⁹ or cytosponge.¹⁰ Interestingly, miR-375, a microRNA that negatively regulates ATG7 expression and autophagy,¹¹ is the most downregulated microRNA in esophageal biopsies

from active EoE subjects.¹² However, additional studies are required to investigate any functional relationship between microRNA, autophagy and EoE and potential autophagy-related biomarkers. Future studies will also assess ATG7 as a prognostic marker interpreted in the context of patient age, disease course and response to therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ATG	Autophagy-related
EoE	Eosinophilic esophagitis
GERD	Gastroesophageal reflux disease
PPI	Proton pump inhibitor
qRT-PCR	quantitative reverse transcription-polymerase chain reaction
ROC	receiver-operating characteristic.

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Study Highlights

What is known

- 1) EoE is a chronic, allergen-mediated disorder resulting in esophageal eosinophilia and dysfunction.
- 2) EoE diagnosis and monitoring are currently limited to endoscopy with biopsies while on high-dose PPI therapy.
- 3) Autophagy is a cellular adaptive homeostatic mechanism with implications in other immune mediated gastrointestinal disorders such as Crohn Disease.

What is new

- 1) ATG7 mRNA is upregulated in active EoE patients when compared to normal, EoE remission and PPI-treated GERD patients.
- 2) ATG7 may serve as a valuable tissue biomarker of active EoE.
- 3) Autophagy and related genes can be explored further to understand the role in EoE and for additional disease biomarkers.

Figure 1. ATG7 may serve as novel tissue biomarker for diagnosis of active EoE inflammation in pediatric patients

(a) qRT-PCR was performed to evaluate ATG7 mRNA expression in esophageal biopsies.
 (b) ROC curve for predicted values from logistic regression analysis of ATG7 adjusted for age in active versus non-active EoE subjects.

Table 1

Clinical Data Summary including disease status with percent of male subjects per group as well as age in years (y) and eosinophil count per high power field (hpf) represented by median and interquartile range (IQR)

Status	Age (y) range, Median (IQR)	Eosinophil count/hpf, Median (IQR)	Male, n (%)
Normal (n=20)	7.33 (4.6 – 15.5)	0.00 (0.0 – 0.0)	19 (95%)
GERD (n=6)	4.33 (1.8 – 7.7)	0.00 (0.0 – 0.0)	4 (67%)
EoE Remission (n=24)	7.50 (4.9 – 9.4)	1.00 (0.0 – 3.5)	20 (83%)
Active EoE (n=27)	11.42 (5.7 – 13.7)	35.00 (25.0 – 50.0)	22 (81 %)