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STAT6 Variants Associate With Relapse of Eosinophilic Esophagitis in Patients Receiving Long-term Proton Pump Inhibitor Therapy

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BACKGROUND & AIMS: **Based on histologic features, variants in *STAT6* are associated with a poor initial response to proton pump inhibitor (PPI) therapy in pediatric patients with eosinophilic esophagitis (EoE). We investigated whether these genetic variants are associated with a poor long-term response in children with EoE who initially responded to PPI therapy.**

METHODS: **We performed a prospective longitudinal cohort study of children ages 2 to 16 years who met the diagnostic criteria for EoE (≥15 eosinophils/high-power field [eos/hpf]), responded to 8 weeks of treatment with 2 mg/kg/d PPI (<15 eos/hpf), and whose dose then was reduced to 1 mg/kg/d PPI (maintenance therapy) for 1 year, at which point biopsy specimens were collected by endoscopy. Genomic DNA was isolated from formalin-fixed paraffin-embedded biopsy tissue and was genotyped for variants of *STAT6*. Remission of inflammation was assessed at eos/hpf thresholds of <15 and ≤5.**

RESULTS: **Among 73 patients who received 1 mg/kg/d PPI maintenance therapy for 1 year, 13 patients (18%) had 6 to 14 eos/hpf, 36 patients (49%) had 5 or fewer eos/hpf, and 24 patients (33%) relapsed to EoE (≥15 eos/hpf). Carriage of any of 3 *STAT6* variants in linkage disequilibrium (r^2 ≥0.8; rs324011, rs167769, or rs12368672) was associated with a 2.3- to 2.8-fold increase in the odds of EoE relapse, and with a 2.8- to 4.1-fold increase in the odds of having 6 to 14 eos/hpf. For rs324011, the odds ratio [95% CI] for relapse was 2.77 [1.11, 6.92]; $P = .029$, and the odds ratio [95% CI] for having 6 to 14 eos/hpf was 3.06 [1.27, 7.36]; $P = .012$.**

CONCLUSIONS: **Pediatric EoE patients who initially respond to PPI therapy and carry *STAT6* variants rs324011, rs167769, or rs12368672 are at increased risk of relapse after 1 year of PPI maintenance therapy.**

Keywords: Esophagus; Biomarker; Response to Treatment; Immune Response.

Recent changes to the eosinophilic esophagitis (EoE) clinical consensus guidelines now support proton pump inhibitor (PPI) medications as a primary therapy for both pediatric and adult EoE.[1] Gutierrez-Junquera et al.[2] previously reported a PPI response rate of 68.6% (PPI–responsive esophageal eosinophilia [PPI-REE], <15 eosinophils/high-power field [eos/hpf]) after 8 weeks of high-dose PPI therapy (twice daily, 1 mg/kg/dose; maximum, 80 mg/d) in children presenting with 15 or more eos/hpf and meeting the diagnostic criteria for EoE. Of the initial responders, it subsequently

Abbreviations used in this paper: **BLRM, binary logistic regression modeling; EoE, eosinophilic esophagitis; eos/hpf, eosinophils per high-power microscope field; EREFS, Eosinophilic Esophagitis Endoscopic Reference Score; mRNA, messenger RNA; OR, odds ratio; PPI, proton pump inhibitor; PPI-REE, proton pump inhibitor responsive eosinophilic esophagitis; RR, rate ratio; SNP, single-nucleotide polymorphism.**

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was found that 70.1%[3] remained in histologic remission after 1 year of low-dose PPI maintenance therapy (once daily, 1 mg/kg/d; maximum, 40 mg/d). Although PPI therapy is effective in treating inflammation associated with EoE, data on the safety of double-dose and long-term PPI treatment in children are scarce.[4] Reports have indicated multiple potential associations between PPI use and comorbidities in children,[5] including an increased risk of respiratory tract[6] or gastrointestinal infections[7] arising from ingestion of orally acquired pathogens that otherwise would be rendered harmless by gastric acid.[8–10] Therefore, among children with EoE, it is important clinically to identify not only the minimal effective PPI dose and duration for a child, but also to identify children who will benefit from low-dose PPI maintenance therapy without the additional risk incurred from endoscopic procedures that will be required if they were to relapse.

Esophageal eosinophilia in EoE is driven by *STAT6*-dependent local expression of eotaxin-3 (*CCL26*), and PPIs can block the chromatin remodeling that is necessary for *STAT6* binding and transcriptional activation of *CCL26*.[11] Individual variability in PPI pharmacokinetics and pharmacodynamics is influenced strongly by genetic variation in *CYP2C19*.[12,13] We have shown previously that carriers of *STAT6* genetic variant rs324011 are 6.1-fold more likely to fail to achieve complete resolution of inflammation than noncarriers and that this effect is compounded in carriers of the *CYP2C19*17* increased function variant.[14] Genetic variants that are associated with response to long-term PPI maintenance therapy for pediatric EoE remain to be characterized. We hypothesized that genetic variants of *STAT6* and *CYP2C19* may influence long-term response to PPI therapy in children with EoE.

What You Need to Know

Background

More than 30% of pediatric patients who are receiving long-term proton pump inhibitor (PPI) maintenance therapy for eosinophilic esophagitis (EoE) relapse. It is not currently possible to identify which patients will relapse.

Findings

Pediatric EoE patients with an initial response to PPI therapy and who carry *STAT6* variants rs324011, rs167769, or rs12368672 are at increased risk of relapse after 1 year of PPI maintenance therapy.

Implications for patient care

Pediatric patients with EoE should be tested for variants in *STAT6* that are associated with a poor response to long-term PPI maintenance therapy.

Methods

Study Participants

Study participants were recruited prospectively to the parent study at 2 pediatric hospitals in Madrid, Spain, between February 2013 and December 2017, as previously described.[2,3] Briefly, children from 2 to 16 years of age who were referred to the pediatric gastroenterology unit with at least 1 symptom possibly related to esophageal dysfunction including heartburn, chest pain, food impaction, abdominal pain, vomiting, regurgitation, dysphagia, and feeding difficulties, in addition to the finding of esophageal eosinophilia (peak value, $\geq$15 eos/0.24 mm^2), were enrolled in the primary study. The study was conducted according to the Declaration of Helsinki. All patients or parents provided their consent to participate in the study. Approvals from the Ethics Committee at the Hospital Universitario Puerta de Hierro-Majadahonda and the Hospital Universitario Severo Ochoa were obtained. Because *CYP2C19* is not expressed in the human liver during infancy,[15] only children ages 2 years or older were included in the present study. After an initial endoscopy with biopsy, participants were treated with PPI (n = 103 esomeprazole, n = 3 lansoprazole, n = 3 omeprazole; twice daily at a target dose of 1 mg/kg/dose, for a total dose of 2 mg/kg/d, up to a maximum dose of 80 mg/d), for 8 weeks (high-dose). Then, a second endoscopy with biopsy was performed while participants still were taking PPI. Seventy-three responders (<15 peak eos/0.24 mm^2) were stepped down to once-daily dosing of 1 mg/kg/d PPI for 1 year (low-dose), at which time a third endoscopy with biopsy was performed. Patient outcomes at the end of the maintenance period have been reported previously.[3] As reflected in Supplementary Figure 1, the dose range across all 73 participants was 0.23 to 1.22 mg/kg/d. One patient received less than 0.5 mg/kg/d. The variation in PPI dose (mg/kg/d) was the result of either reaching the maximum daily dose of 40 mg, or a result of trying to achieve their target dose while being restricted to prescribing available esomeprazole tablet preparations of 20 and 40 mg. Additional details about the current cohort can be found in the Supplementary Methods section.

Histologic Definition of Disease and Response to Proton Pump Inhibitor

Biopsies were performed (at least 2 from the distal esophagus and 2 from the proximal-mid-esophagus) according to the guidelines for diagnosis and monitoring of EoE.[1,16] All biopsies were targeted to areas with abnormal endoscopic findings, if present. After fixation in 10% buffered formalin and staining with H&E, eosinophil counts from single high-power microscope fields corresponding to an area of 0.24 mm^2 were recorded. Esophageal eosinophilia was defined as having a peak

Table 1. Patient Characteristics

	<15 eos/hpf (n = 49)[a]	≥15 eos/hpf (n = 24)[a]	*P* value[b]
Therapy			
Esomeprazole	45 (91.8)	23 (96)	1
PPI maintenance dose, *mg/kg/d*	0.93 (0.6)	0.91 (0.65)	.62
Characteristics at diagnosis			
Caucasian	48 (98)	23 (95.8)	1
Male	34 (69.4)	20 (83.3)	.26
Age at diagnosis, *y*	10.37 (5.46)	9.58 (6.43)	.41
Height (z-score)	-0.51 (2.53)	-0.32 (2.82)	.46
Weight (z-score)	-0.54 (1.58)	-0.43 (1.6)	.62
History at diagnosis			
Allergic rhinitis	22 (44.9)	8 (34.8)	.45
Asthma	17 (34.7)	13 (54.2)	.13
Atopy	10 (20.4)	6 (25)	.77
Food allergies	10 (20.4)	10 (41.7)	.09
Symptoms at diagnosis			
Abdominal pain	35 (71.4)	14 (58.3)	.30
Dysphagia	18 (36.7)	12 (50)	.32
Food refusal	14 (28.6)	7 (29.2)	1
Heartburn	15 (30.6)	5 (20.8)	.42
Impaction	10 (20.4)	9 (37.5)	.16
Regurgitation/vomiting	13 (26.5)	7 (29.2)	1
Retrosternal pain	11 (22.4)	4 (16.7)	.76
EREFS			
EREFS at diagnosis	4 [3–4]	4 [3–4]	.49
Edema at the beginning of the maintenance period	0 [0–1]	1 [1–1]	**.001**
Furrows at the beginning of the maintenance period	0 [0–0]	0 [0–1]	.07
EREFS at the beginning of the maintenance period	1 [1–2]	2 [2–3]	**<.001**
Endoscopy			
PPI-REE, <15 and >5 eos/hpf, end of high-dose period	16 (32.7)	13 (54.2)	.13
PPI-REE, ≤5 eos/hpf, end of high-dose period	33 (67.3)	11 (22.4)	.13
Peak eos/hpf at diagnosis	46 [35–72]	55 [30–80]	.64
Delta peak eos/hpf over the high-dose period	-44 [-70 to -36]	-50 [-77 to -25]	.84
Peak eos/hpf at end of high-dose period	2 [0–3]	7 [0–9]	.15

eos/hpf, eosinophils per high-powered microscope field; EREFS, Eosinophilic Esophagitis Endoscopic Reference Score; PPI-REE, proton pump inhibitor responsive eosinophilic esophagitis.

[a]Participant counts are reported as n (%N); a Box Cox transformation is applied to continuous data and the back transformed means (SD) are reported; count data (other than participant count) is reported as median [95% CI].

[b]Reported value is for the Fisher exact test, 2-sided (participant counts), the Welch t test, 2-sided (continuous data), or negative binomial regression (count data). Values in bold font indicate characteristicts that are significantly different between groups. No correction has been made for multiple testing.

eosinophil count of 15 or more per high-power field in 1 or more esophageal biopsy specimens at baseline. After 8 weeks of high-dose PPI therapy, response was defined as fewer than 15 eos/hpf on all esophageal biopsy specimens obtained during the follow-up upper gastrointestinal endoscopy. After 1 year of PPI maintenance therapy, response was assessed at thresholds of <15 eos/hpf and ≤5 eos/hpf.

Genotyping

Genomic DNA was isolated from formalin-fixed paraffin-embedded sections of esophageal biopsy tissue,[17] and genotyping reactions were conducted as previously described.[17] The *CYP2C19* single-nucleotide polymorphisms (SNPs) investigated and assays used were as previously described.[14,17] The *STAT6* SNPs investigated and the TaqMan assays used (Applied Biosystems, Waltham, MA) were as follows: rs1059513 (C___7480847_10), rs324011 (C___620399_10), rs167769 (C___620401_20), and rs12368672 (C__31186828_10). Genotype counts, SNP frequencies, and Hardy–Weinberg equilibrium *P* values are shown in Supplementary Table 1. SNPs rs324011, rs167769, and rs12368672 were in linkage disequilibrium (r^2, >0.8) both in our population and in the 1000 Genomes phase 3 data set.[18]

Statistical Analysis

Analyses were conducted in R base versions 3.5.1 (2018)[19] and are described in the Supplementary Materials. Inflation of type 1 error through multiple

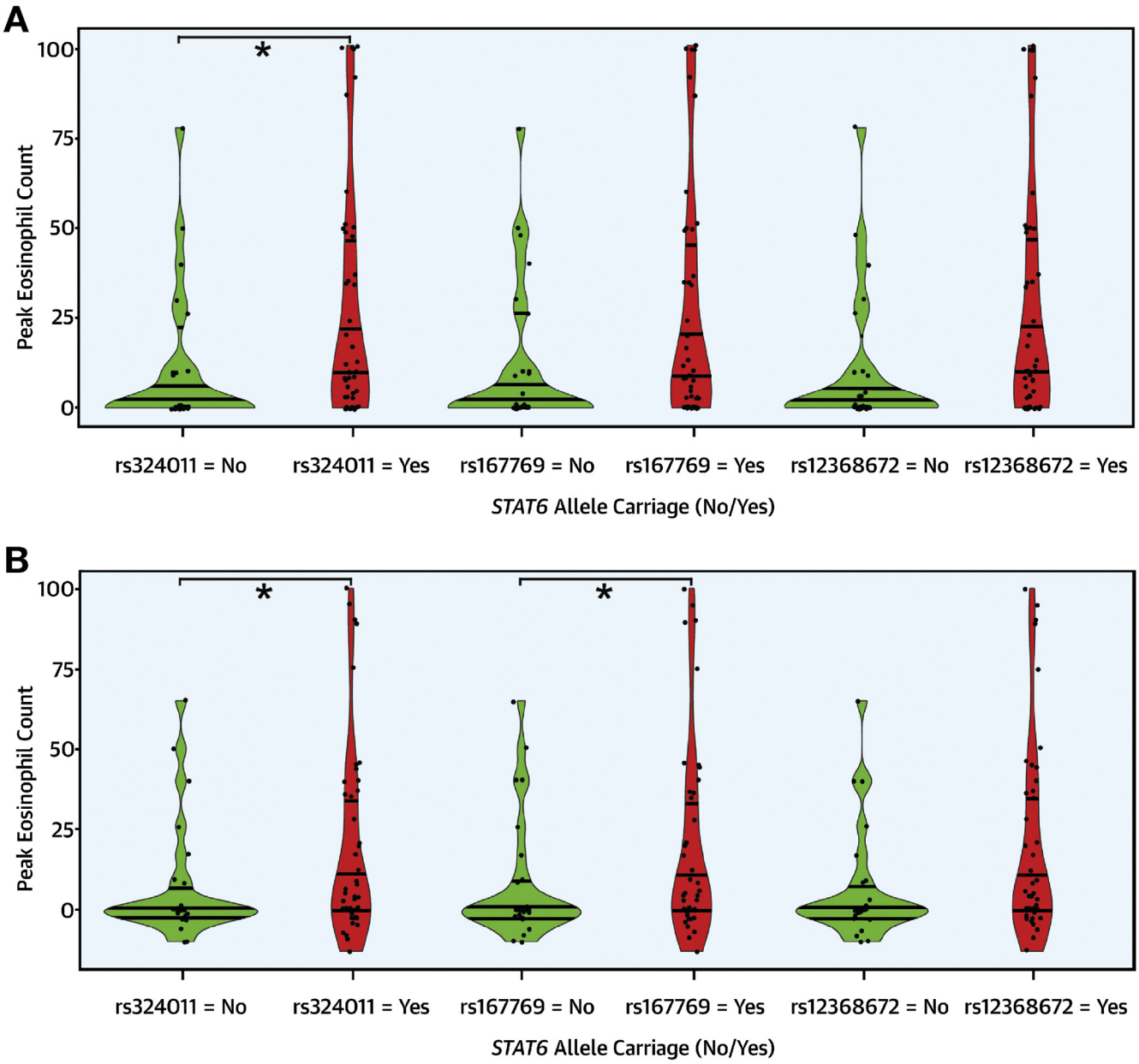

Figure 1. Distribution of eosinophil counts at the initiation of maintenance therapy and the change in peak eosinophil counts during maintenance therapy differ by *STAT6* genotype. Violin plots of (*A*) peak eosinophil count at the end of the 1-year proton pump inhibitor (PPI) maintenance period or (*B*) peak eosinophil count change (end minus beginning) during the 1-year maintenance period, stratified by *STAT6* genotype (dominant genetic model). *Black points* indicate eos counts for individuals and have been jittered on the x-axis to reduce overlap. *Black lines* within shaded regions indicate (from the bottom) first, second, and third quantiles based on the density distribution. *Horizontal brackets with asterisks* indicate that the distributions are significantly different ($P \leq .05$).

testing has been addressed by correction of reported *P* values using the Bonferroni method.[20]

Results

The consort diagram for this study is shown in Supplementary Figure 1. The baseline characteristics of study patients stratified by clinical outcome are detailed in Table 1. Overall, the PPI dose for maintenance therapy ranged from 0.23 to 1.22 mg/kg/d, and 67% of participants scored fewer than 15 eos/hpf at their 1 year follow-up evaluation. Responders (<15 eos/hpf) and nonresponders (≥15 eos/hpf) received similar mean PPI doses. Patients whose inflammation eventually would relapse while on maintenance therapy had a higher median Eosinophilic Esophagitis Endoscopic Reference Score (EREFS)[21] after completion of the initial 8 weeks of high-dose PPI therapy (≥15 eos/hpf, median EREFS [95% CI], 2 [2,3] vs <15 eos/hpf, 1 [1,2]; P <.001), driven primarily by increased edema (≥15 eos/hpf: odds ratio [OR] [95% CI], 10.2 [2.53, 41.16]; $P = .001$) and furrows (≥15 eos/hpf: OR [95% CI], 3.53 [0.92, 13.51];

$P = .065$), in patients who eventually would relapse. Binary logistic regression modeling (BLRM) with race, sex, age, PPI dose, and PPI type as covariates found that EREFS at this point, predicted histologic relapse of inflammation after 1 year of maintenance therapy (≥15 eos/hpf: OR [95% CI], 3.70 [1.64, 8.37]; $P = .002$), meaning that for every 1-point increase in EREFS, patients were 3.7 times more likely to experience histologic relapse. EREFS at the beginning of the high-dose PPI therapy period did not predict response to maintenance therapy. From BLRM, we found that patients who scored 5 or fewer eos/hpf after the initial 8 weeks of high-dose PPI therapy were greater than 3-fold more likely to remain in remission during maintenance therapy than individuals who initially scored fewer than 15 (<15 eos/hpf: OR [95% CI], 3.06 [1.04, 9.01]; $P = .042$) and were 4.5-fold more likely to remain at 5 or fewer eos/hpf after 1 year of maintenance therapy (≤5 eos/hpf: OR [95% CI], 4.51 [1.54, 13.15]; $P = .006$).

We previously reported that carriage of *STAT6* variants rs324011, rs167769 and rs12368672 was associated with an increased eosinophil count in the distal esophagus, relative to noncarriers, on biopsy samples obtained before initiating PPI therapy,[14] while carriage of *STAT6* variant rs1059513 was associated with decreased eosinophil counts in the distal esophagus. Therefore, we analyzed this group of SNPs for association with outcome of PPI maintenance therapy. Genotype counts, SNP frequencies, and Hardy–Weinberg equilibrium *P* values for the variants examined in the study population are shown in Supplementary Table 1.

The distributions of both peak esophageal eosinophil counts at the end of 1 year of maintenance therapy over all regions sampled (distal and medium proximal) and the change in peak eosinophil counts over the maintenance period were qualitatively different between noncarriers and carriers of rs324011, rs167769, and rs12368672 (Figure 1*A* and *B*, respectively). In particular, the quantiles of eosinophil count distribution density for both metrics were skewed to larger values in carriers relative to noncarriers. By using negative binomial regression (dominant model, covariates as described earlier), we found that carriage of rs324011, rs167769, and rs12368672 tended to be associated with a 2.60- to 3.12-fold increase in median peak esophageal eosinophil count at the end of 1 year of maintenance therapy relative to noncarriers (Table 2) (rs324011, median change in peak eos/hpf [95% CI], +9 [-2,20] eos/hpf; rate ratio (RR) [95% CI], 3.12 [1.25, 7.76]; $P = .028$). The same group of SNPs also tended to be associated with a 1.49- to 1.60-fold increase in change in peak eosinophil counts over the 1-year maintenance period (Table 2) (rs324011, median change in peak eos/hpf [95% CI], +4 [0,17] eos/hpf; RR [95% CI], 1.60 [1.12, 2.30]; $P = .022$). Although individuals who carry rs1059513 had lower peak eosinophil counts at the end of 1 year of maintenance therapy than noncarriers (median change in peak eos/hpf [95% CI], -5 [-19,9]), the result was not significant in this population.

Finally, we tested for associations between carriage of *STAT6* and/or *CYP2C19*17* variants and outcome of PPI maintenance therapy (Figure 2). In BLRM, examining the association between *STAT6* variant and scoring 15 or more eos/hpf after 1 year of maintenance therapy (additive genetic model, covariates as described earlier), individuals who were carriers of rs324011, rs167769, and rs12368672 had a 2.32- to 2.80-fold increased odds of scoring 15 or more eos/hpf after 1 year of PPI maintenance therapy than noncarriers (rs324011, ≥15 eos/hpf OR [95% CI], 2.77 [1.11, 6.92]; $P = .029$). When considering individuals who experienced some level of

Table 2. Esophageal Eosinophil Counts Are Associated With Carriage of *STAT6* Variants

SNP	Outcome[a]	Genotype counts, dom = 0/1[b]	eos/hpf, dom = 0, median [95% CI]	eos/hpf, dom = 1, median [95% CI]	RR [95%CI]	*P* value[c]
rs1059513	Post-PPI peak eos/hpf	55/18	8 [1–10]	3 [0–26]	0.71 [0.25–2.05]	1
rs324011		26/47	0 [0–9]	9 [3–20]	3.12 [1.25–7.76]	**.028**
rs167769		28/45	0 [0–9]	9 [3–17]	2.73 [1.10–6.74]	.060
rs12368672		26/47	0.5 [0–4]	10 [3–20]	2.60 [1.04–6.50]	.082
rs1059513	Δ peak eos/hpf	55/18	0 [0–6]	2 [0–26]	0.96 [0.63–1.46]	1
rs324011		26/47	0 [-1 to 0]	4 [0–17]	1.60 [1.12–2.30]	**.022**
rs167769		28/45	0 [-1 to 0]	4 [0–12]	1.55 [1.08–2.22]	**.032**
rs12368672		26/47	0 [-1 to 1]	4 [0–17]	1.49 [1.03–2.14]	.064

dom, dominant genetic model; eos/hpf, eosinophils per high-powered field; PPI, proton pump inhibitor; RR, rate ratio; SNP, single-nucleotide polymorphism.
[a]Peak value was the highest recorded value from all biopsy specimens in all regions sampled.
[b]Genetic model coding, dominant: carriage of 0 copies of the SNP is coded as 0, carriage of 1 or 2 copies of the SNP is coded as 1.
[c]Reported value is from negative binomial regression modeling with eosinophil counts as the dependent variable and genotype counts as the independent variable, adjusted for race, age, sex, PPI dose (mg/kg/d), and type of PPI.

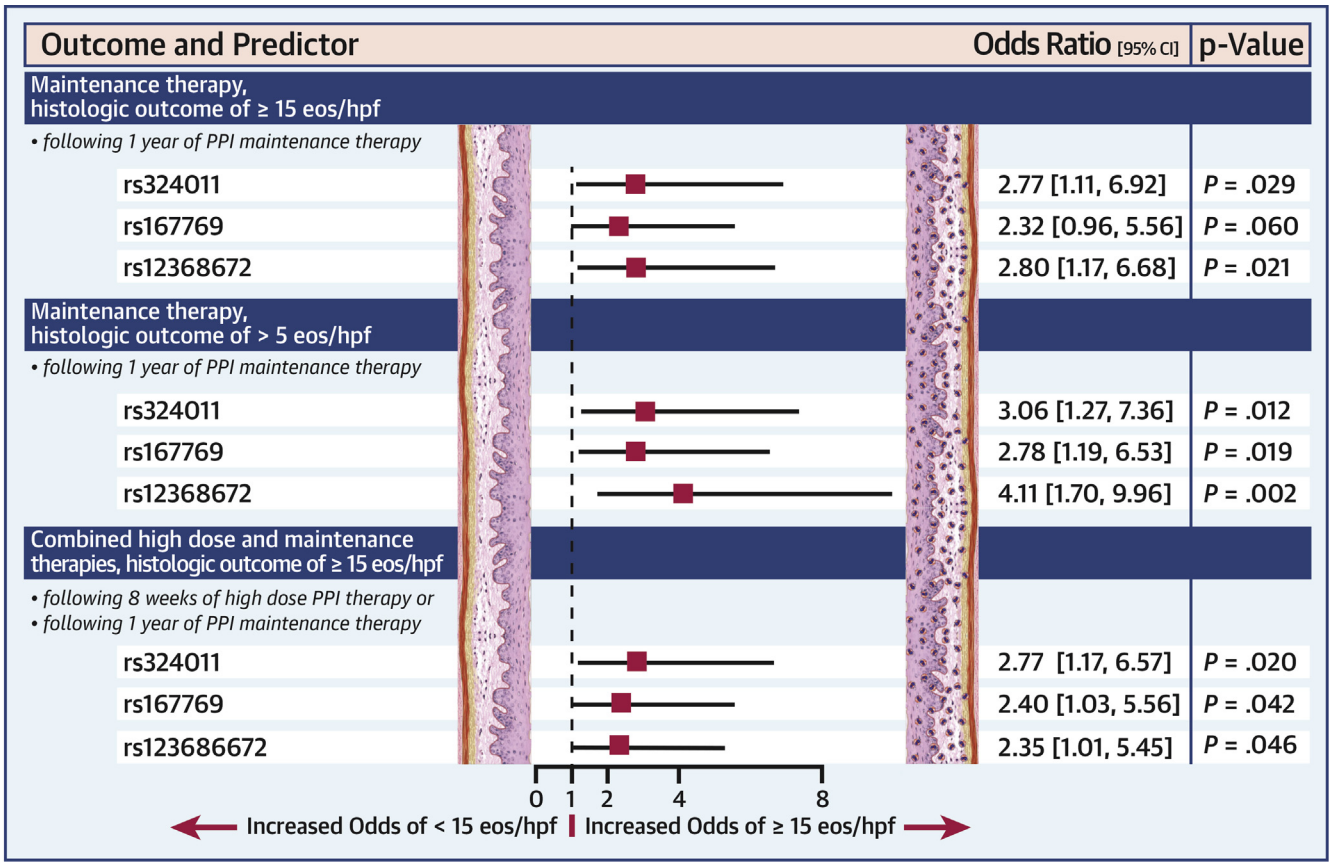

Figure 2. Histologic relapse of eosinophilic esophagitis (EoE) after 1 year of proton pump inhibitor (PPI) maintenance therapy was associated with carriage of *STAT6* variants. Binary logistic regression modeling (BLRM) of *STAT6* variants as predictors of histologic relapse (≥15 eosinophils [eos]/0.24 mm^2, *top*) or (>5 eos/0.24 mm^2, *middle*), over the 1-year maintenance therapy period and over both the initial 8-week, high-dose therapy and 1-year maintenance therapy periods combined (≥15 eos/0.24 mm^2, *bottom*). The odds of failing to respond to therapy (≥15 eos/0.24 mm^2) increase toward the *right*. All models include race, sex, age, PPI dose, and PPI type as covariates. hpf, high-powered field.

inflammation less than complete relapse and greater than complete remission (<15 and >5 eos/hpf) after 1 year of maintenance therapy, carriage of rs324011, rs167769 and rs12368672 increased the OR 2.78- to 4.11-fold (rs324011: <15 and >5 eos/hpf OR [95% CI], 3.06 [1.27, 7.36; $P = .012$). When considering both therapy periods together (8 weeks + maintenance), individuals who carry rs324011, rs167769 and rs12368672 have a 2.6- to 2.8-fold increased odds of failing PPI therapy at some point (rs324011: ≥15 eos/hpf OR [95% CI], 2.77 [1.17, 6.57; $P = .020$). Interestingly, we did not find significant associations between carriage of *CYP2C19*17* and outcome of PPI maintenance therapy, and we did not see interactions between rs324011, rs167769, rs12368672 and *CYP2C19*17* in this population.

Discussion

In this study, we identify novel associations between common genetic variants in *STAT6* and histologic relapse of eosinophilic inflammation in pediatric patients who are receiving 1 mg/kg/d PPI therapy for long-term maintenance of inflammation remission. Specifically, carriage of any 1 of 3 *STAT6* variants in linkage disequilibrium (rs324011, rs167769, or rs12368672) is associated with a 2.60- to 3.12-fold increase in peak eosinophil count at the end of 1 year of maintenance therapy; a 1.49- to 1.60-fold increase in the difference between peak eosinophil counts at the end and at the beginning of 1 year of maintenance therapy; and a 2.32- to 2.80-fold increase in the odds of histologic relapse to EoE (≥15 eos/hpf) during the maintenance period. This is consistent with our previous findings after 8 weeks of high-dose PPI therapy that carriage of the same group of *STAT6* variants was associated with a 6.1-fold increased odds of failing to achieve complete PPI-REE[14] (≤5 eos/hpf). The study found an association between *STAT6* variants and response to long-term PPI maintenance therapy for EoE and may have implications for clinical management.

In contrast to our previous findings in the initial 8-week, high-dose PPI study that showed the influence of *CYP2C19*17* within the interquartile range of all PPI dosages given and an interaction between *CYP2C19*17* and *STAT6* rs324011, we saw neither association in the current study. This finding also was contrary to a report

by Molina-Infante et al,[22] suggesting that *CYP2C19*17* predicted failure of long-term PPI maintenance therapy (predominantly omeprazole) in an adult retrospective cohort study. It is possible that the predominant PPI type (eg, omeprazole) and dose combinations used by Molina-Infante et al[22] were more sensitive to the effects of *CYP2C19*17* than those used in our studies.[2,3] This interpretation is strengthened by evidence-based guidelines published by the Dutch Pharmacogenetics Working Group,[23] which recommend increasing the dose of omeprazole, lansoprazole, and pantoprazole by 3-, 4-, and 5-fold, respectively, in carriers of *CYP2C19*17* to maintain therapeutic efficacy for acid suppression, while refraining from making a similar recommendation for esomeprazole. This is consistent with estimated relative potencies of 0.23, 0.9, and 1.6 for pantoprazole, lansoprazole, and esomeprazole in general, compared with omeprazole, based on mean 24-hour gastric pH measurements.[24] Although there is limited evidence to support a role for gastric acid suppression in the mechanism of PPI-REE, PPI dose dependency has been established for the anti-inflammatory mechanism through which PPI may be functioning in EoE.[11] Because the majority of patients in our studies receive esomeprazole and given that the AUC of esomeprazole is almost 2-fold greater than an equivalent dose of omeprazole,[25] we would expect to see decreased influence of *CYP2C19*17* in our studies relative to studies that predominately use omeprazole, lansoprazole, or pantoprazole. Consequently, we suspect that the current work is underpowered to see the influence of *CYP2C19*17* or its interaction with rs324011.

The association we characterize between *STAT6* variants and response to PPI therapy for long-term maintenance of inflammation remission for EoE is consistent with the known role of *STAT6* as a critical mediator of the interleukin 4 and interleukin 13 cytokine signaling pathways. Rs324011 is located in intron 2 of the *STAT6* gene. In vitro studies have suggested that the major allele variant of rs324011 (C) is associated with transcriptional silencing of *STAT6*, while the minor allele variant (T) creates a functional nuclear factor-κB binding site that is associated with both enhanced STAT6 pre–messenger RNA (mRNA) expression and increased aberrant splicing through intron retention.[26] Analysis of gene expression quantitative trait loci in B cells and monocytes shows that rs324011 (T) dose-dependently is associated with increased STAT6 mRNA expression when using a probe that tags the 3' untranslated region.[27] Genotype tissue expression[28] analysis identifies rs324011 (T) as a significant STAT6 pre-mRNA splicing quantitative trait loci that is associated with aberrant splicing through intron retention in a tissue-dependent manner. Interestingly, esophageal mucosa is among the tissues in which aberrant splicing of STAT6 pre-mRNA is greatest. Exactly how the diverse biological phenotypes associated with rs324011, or other genetic variants in linkage disequilibrium with rs324011, interact to increase esophageal eosinophil counts in carriers of rs324011 remains to be determined.

This study had several limitations including small sample size, variation in PPI dose, length of therapy, and the potential for additional genetic variants identified in previous genome-wide association studies[29,30] to act as confounders and influence histologic outcome of PPI therapy. Because of minor heterogeneity in race (72 European Caucasians, 1 African American, and 1 Arab) and type of PPI (68 esomeprazole, 3 omeprazole, and 2 lansoprazole), corrections for these covariates were included in our regression analyses.

Conclusions

Carriage of *STAT6* variants rs324011, rs167769 or rs12368672 increase the odds that pediatric patients will fail PPI therapy for EoE at some point during either the initial 8-week, high-dose therapy or during the subsequent 1 year of low-dose maintenance therapy. Large prospective clinical studies are needed to fully characterize which factors contribute to PPI response variability (20%–70%[4]) including PPI type, PPI dose, and individual genetics. EoE patients may benefit from personalized, genotype-guided therapy selection, and/or dose optimization.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2020.08.020.

References

1. Lucendo AJ, Molina-Infante J, Arias A, et al. Guidelines on eosinophilic esophagitis: evidence-based statements and recommendations for diagnosis and management in children and adults. United European Gastroenterol J 2017;5:335–358.
2. Gutierrez-Junquera C, Fernandez-Fernandez S, Cilleruelo ML, et al. High prevalence of response to proton-pump inhibitor treatment in children with esophageal eosinophilia. J Pediatr Gastroenterol Nutr 2016;62:704–710.
3. Gutierrez-Junquera C, Fernandez-Fernandez S, Cilleruelo ML, et al. Long-term treatment with proton pump inhibitors is effective in children with eosinophilic esophagitis. J Pediatr Gastroenterol Nutr 2018;67:210–216.
4. Gutierrez-Junquera C, Fernandez-Fernandez S, Cilleruelo ML, et al. The role of proton pump inhibitors in the management of pediatric eosinophilic esophagitis. Front Pediatr 2018;6:119.
5. De Bruyne P, Ito S. Toxicity of long-term use of proton pump inhibitors in children. Arch Dis Child 2018;103:78–82.
6. Writing Committee for the American Lung Association Asthma Clinical Research Centers, Holbrook JT, Wise RA, et al. Lansoprazole for children with poorly controlled asthma: a randomized controlled trial. JAMA 2012;307:373–381.

7. Tjon JA, Pe M, Soscia J, et al. Efficacy and safety of proton pump inhibitors in the management of pediatric gastroesophageal reflux disease. Pharmacotherapy 2013;33:956–971.
8. Freedberg DE, Toussaint NC, Chen SP, et al. Proton pump inhibitors alter specific taxa in the human gastrointestinal microbiome: a crossover trial. Gastroenterology 2015;149:883–885 e889.
9. Imhann F, Bonder MJ, Vich Vila A, et al. Proton pump inhibitors affect the gut microbiome. Gut 2016;65:740–748.
10. Jackson MA, Goodrich JK, Maxan ME, et al. Proton pump inhibitors alter the composition of the gut microbiota. Gut 2016; 65:749–756.
11. Zhang X, Cheng E, Huo X, et al. Omeprazole blocks STAT6 binding to the eotaxin-3 promoter in eosinophilic esophagitis cells. PLoS One 2012;7:e50037.
12. Lima JJ, Franciosi JP. Pharmacogenomic testing: the case for CYP2C19 proton pump inhibitor gene-drug pairs. Pharmacogenomics 2014;15:1405–1416.
13. Franciosi JP, Mougey EB, Williams A, et al. Association between CYP2C19 extensive metabolizer phenotype and childhood anti-reflux surgery following failed proton pump inhibitor medication treatment. Eur J Pediatr 2018; 177:69–77.
14. Mougey EB, Williams A, Coyne AJK, et al. CYP2C19 and STAT6 variants influence the outcome of proton pump inhibitor therapy in pediatric eosinophilic esophagitis. J Pediatr Gastroenterol Nutr 2019;69:581–587.
15. Koukouritaki SB, Manro JR, Marsh SA, et al. Developmental expression of human hepatic CYP2C9 and CYP2C19. J Pharmacol Exp Ther 2004;308:965–974.
16. Dellon ES, Gonsalves N, Hirano I, et al. ACG clinical guideline: evidenced based approach to the diagnosis and management of esophageal eosinophilia and eosinophilic esophagitis (EoE). Am J Gastroenterol 2013;108:679–692; quiz 693.
17. Franciosi JP, Mougey EB, Williams A, et al. Association between CYP2C19*17 alleles and pH probe testing outcomes in children with symptomatic gastroesophageal reflux. J Clin Pharmacol 2018;58:89–96.
18. 1000 Genomes Project Consortium, Auton A, Brooks LD, et al. A global reference for human genetic variation. Nature 2015; 526:68–74.
19. R: a language and environment for statistical computing [computer program]. Vienna, Austria: R Foundation for Statistical Computing, 2008.
20. Dunn OJ. Estimation of the means for dependent variables. Ann Math Stat 1958;29:1095–1111.
21. Hirano I, Moy N, Heckman MG, et al. Endoscopic assessment of the oesophageal features of eosinophilic oesophagitis: validation of a novel classification and grading system. Gut 2013; 62:489–495.
22. Molina-Infante J, Rodriguez-Sanchez J, Martinek J, et al. Long-term loss of response in proton pump inhibitor-responsive esophageal eosinophilia is uncommon and influenced by CYP2C19 genotype and rhinoconjunctivitis. Am J Gastroenterol 2015;110:1567–1575.
23. Group DPW. The Dutch Pharmacogenetics Working Group (DPWG) pharmacogenomic guidelines. 2019. Available at: https://www.knmp.nl/downloads/pharmacogenetic-recommendations-november-2019.pdf. Accessed January 8, 2020.
24. Kirchheiner J, Glatt S, Fuhr U, et al. Relative potency of proton-pump inhibitors-comparison of effects on intragastric pH. Eur J Clin Pharmacol 2009;65:19–31.
25. Lind T, Cederberg C, Ekenved G, et al. Effect of omeprazole–a gastric proton pump inhibitor–on pentagastrin stimulated acid secretion in man. Gut 1983;24:270–276.
26. Schedel M, Frei R, Bieli C, et al. An IgE-associated polymorphism in STAT6 alters NF-kappaB binding, STAT6 promoter activity, and mRNA expression. J Allergy Clin Immunol 2009; 124(583–589):589 e581–589 e 586.
27. Fairfax BP, Makino S, Radhakrishnan J, et al. Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. Nat Genet 2012;44:502–510.
28. The Genotype-Tissue Expression (GTEx) Project J. Lonsdale, J. Thomas, M. Salvatore et al. The Genotype-Tissue Expression (GTEx) project, *Nat Genet* **45**, 2013, 580–585. https://doi.org/10.1038/ng.2653**.** The data used for the analyses described in this manuscript were obtained from the GTEx Portal on 12/23/2019.
29. Sleiman PM, Wang ML, Cianferoni A, et al. GWAS identifies four novel eosinophilic esophagitis loci. Nat Commun 2014;5:5593.
30. Rothenberg ME, Spergel JM, Sherrill JD, et al. Common variants at 5q22 associate with pediatric eosinophilic esophagitis. Nat Genet 2010;42:289–291.

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The authors disclose no conflicts.

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Supplementary Methods

Relationship of the Current Cohort to Those of Our Earlier Studies

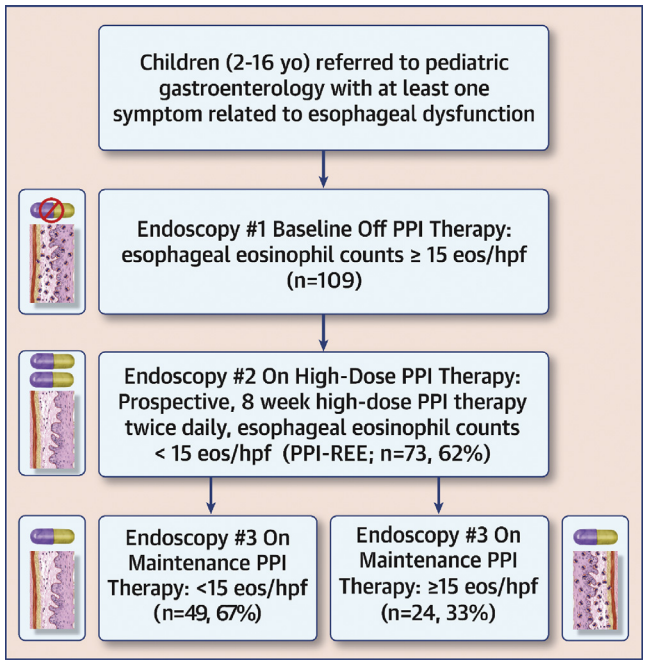
In the initial study (C. Gutierrez-Junquera, S. Fernandez-Fernandez, M.L. Cilleruelo, et al., High prevalence of response to proton-pump inhibitor treatment in children with esophageal eosinophilia, J Pediatr Gastroenterol Nutr 62, 2016, 704–710.), 56 patients were recruited between February 2013 and April 2015, 5 of whom subsequently were excluded (51 patients met inclusion criteria). These patients received 2 mg/kg/d PPI therapy for 8 weeks (induction phase). Between May 2015 and August 2017, an additional 58 patients completed the induction phase and were added to the initial group of 51 patients for a total of 109 patients. Of these 109 patients, 72 achieved histologic remission after the induction phase and were stepped-down to 1 mg/kg/d PPI therapy for 12 months (maintenance phase). Of these 72 patients, 2 were lost to follow-up evaluation, 1 refused a follow-up endoscopy, and 12 had their follow-up visits before completing 12 months of maintenance therapy. The remaining 57 patients were the subject of the second study (C. Gutierrez-Junquera, S. Fernandez-Fernandez, M.L. Cilleruelo, et al., Long-term treatment with proton pump inhibitors is effective in children with eosinophilic esophagitis, J Pediatr Gastroenterol Nutr 67, 2018, 210–216.). In our third study (E.B. Mougey, A. Williams, A.J.K. Coyne, et al., CYP2C19 and STAT6 variants influence the outcome of proton pump inhibitor therapy in pediatric eosinophilic esophagitis, J Pediatr Gastroenterol Nutr 69, 2019, 581–587. 92 participants), we examined the 57 induction phase responders from our second study together with the 37 nonresponders, 2 of whom were excluded because they received swallowed steroids in addition to PPIs. In the present study, the 57 participants of the second study were combined with an additional 17 patients who completed the maintenance phase between September 2017 and February 2019, for a total of 73 participants.

Statistical Methods

Analyses were conducted in R base versions 3.5.1 (2018).[1] Continuous variables were transformed using the powerTransform function of the R package car.[2] The statistical test used was dependent on the data distribution. A 2-sided Fisher exact test (exact *P* value) was used for comparison of proportions in count data. A 2-sided Wilcoxon rank-sum test (exact *P* value) was used to determine whether 2 independent samples were selected from populations having the same distribution. Negative binomial regression from the R statistical package MASS,[3] was used with auto-optimization of the dispersion parameter to assess relationships between independent variables and count-dependent variables. Binary logistic regression was used to assess relationships between independent variables and binary-dependent variables. Plots were produced using the function ggplot from the R statistical package ggplot2.[4] Forest plots were prepared with the R package forestplot.[5] When differences between values with CIs were calculated, the MOVER-D method[6] was used to propagate imprecision. Inflation of type 1 error through multiple testing has been addressed by correction of reported *P* values using the method of Bonferroni.[7]

Supplementary References

1. R: A language and environment for statistical computing, [computer program]. Vienna, Austria: R Foundation for Statistical Computing, 2008.
2. Fox JW. An {R} companion to applied regression. [computer program], 2nd ed. Thousand Oaks, CA: Sage, 2011.
3. Venables WNR. Modern applied statistics with S. New York: Springer, [computer program], 2002.
4. H. Wickham. ggplot2: Elegant Graphics for Data Analysis, [computer program], 2016. Springer-Verlag New York
5. M. Gordon, T. Lumley. (2020). forestplot: Advanced Forest Plot Using 'grid' Graphics, [computer program], 2020. R package version 1.10.
6. R.G. Newcombe. Confidence Intervals for Proportions and Related Measures of Effect Size, 2012, CRC Press, Taylor & Francis Group, Boca Raton, FL
7. Dunn OJ. Estimation of the means for dependent variables. Ann Math Stat 1958;29:1095–1111.



Supplementary Figure 1. Consort diagram. Endoscopy #1 was performed for an initial diagnosis. Endoscopy #2 was performed after the initial 8-week, high-dose therapy period. Endoscopy #3 was performed at the end of the 1-year maintenance period. Proton pump inhibitor (PPI) dosages were as follows: during the initial 8 weeks, patients received two 1 mg/kg PPI doses per day, up to a maximum of 80 mg/d; for 1-year maintenance therapy, patients received one 1 mg/kg PPI dose per day, up to a maximum of 40 mg/d. Patient counts and percentages of the total count are reported as follows: n = xx; N%. eos, eosinophils; hpf, high-powered field (0.24 mm^2); PPI-REE, PPI-responsive eosinophilic esophagitis; yo, years old.

Supplementary Table 1. SNPs, Minor Allele Frequency, and Hardy–Weinberg Equilibrium

				<15 eos/hpf	≥15 eos/hpf	
SNP	Position	MAF	HWE	n, MM/mM/mm	n, MM/mM/mm	*P* value[a]
*CYP2C19*2* rs4244285	chr10:94781609	0.199	0.721	35/14/0	11/11/2	.022
*CYP2C19*17* rs12248560	chr10:94761650	0.171	1	34/13/2	16/8/0	.716
STAT6 rs1059513	chr12:57095676	0.130	1	37/11/1	18/6/0	1
***STAT6* rs324011**	chr12:57108149	0.377	0.321	21/24/4	5/15/4	.162
***STAT6* rs167769**	chr12:57109742	0.363	0.612	22/23/4	6/14/4	.214
***STAT6* rs12368672**	chr12:57118437	0.390	0.805	21/24/4	5/13/6	.074

NOTE. Variants in bold are in linkage disequilibrium ($r^2 \geq 0.8$).
chr, chromosome; eos/hpf, eosinophils per high-power field; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; MM, major allele homozygote; mM, minor allele heterozygote; mm, minor allele homozygote; SNP, single-nucleotide polymorphism.
[a]The reported *P* value is for the Fisher exact test, 2-sided, without correction for multiple testing.