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CYP2C19 and STAT6 Variants Influence the Outcome of Proton Pump Inhibitor Therapy in Pediatric Eosinophilic Esophagitis

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ABSTRACT

Objective: Proton pump inhibitors (PPIs) are an effective treatment for eosinophilic esophagitis (EoE); however, only 30% to 60% of patients respond. Common genetic variants in *CYP2C19* and *STAT6* associate with PPI plasma concentration and magnitude of inflammatory response, respectively. Our objective was to determine if genetic variation in the genes for *CYP2C19* and *STAT6* influence differentiation between PPI responsive esophageal eosinophilia versus PPI nonresponsive EoE (PPI-REE, PPI-nonresponsive EoE).

Methods: Genomic DNA was isolated from 92 esophageal tissue biopsies collected from participants of a prospective clinical trial of high-dose PPI therapy for esophageal eosinophilia in children.

Results: Of the 92 patients examined, 57 (62%) were PPI-REE and 35 (38%) were PPI-nonresponsive EoE. Forty-six of the 92 patients were further characterized by pH probe monitoring; there was no association between reflux index and carriage of *CYP2C19**17 ($P = 0.35$). In children who received a PPI dose between ≥ 1.54 and ≤ 2.05 mg/kg/day, binary logistic regression modeling showed that carriage of *CYP2C19**17 associated with PPI-nonresponsive EoE (odds ratio (OR) [95% confidence interval (CI)] = 7.71 [1.21, 49.11], $P = 0.031$). Carriage of *STAT6* allelic variant rs1059513 predicts PPI-REE (OR [95% CI] = 6.16 [1.44, 26.4], $P = 0.028$), whereas carriage of *STAT6* rs324011 synergizes with *CYP2C19**17 to predict PPI-nonresponsive EoE (rs324011 OR [95% CI] = 5.56 [1.33, 20.72], $P = 0.022$; *CYP2C19**17 OR [95% CI] = 8.19 [1.42, 50.57], $P = 0.023$).

Conclusions: Common variants in *CYP2C19* and *STAT6* associate with a PPI-nonresponsive EoE outcome of PPI therapy for esophageal eosinophilia suggesting that response rates may be improved by adopting a genotype-guided approach to PPI dosing.

Key Words: esophagus, genotype guided, inflammation, pharmacogenetics, proton pump inhibitor-nonresponsive eosinophilic esophagitis, proton pump inhibitor-responsive esophageal eosinophilia

(*JPGN* 2019;69: 581–587)

Received September 15, 2018; accepted August 11, 2019.

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What Is Known

- Proton pump inhibitors are an effective treatment for esophageal eosinophilia with a variable response rate of 30% to 60%.
- Proton pump inhibitor pharmacodynamics are strongly influenced by genetic variation in *CYP2C19*.
- *STAT6* genetic variants associate with eosinophilic esophagitis.

What Is New

- Carriers of *CYP2C19**17 are more likely to fail proton pump inhibitor therapy for esophageal eosinophilia within a defined dose range.
- Different *STAT6* genetic variants associate with pre-proton pump inhibitor eosinophil counts and a proton pump inhibitor-responsive esophageal eosinophilia outcome.
- *STAT6* rs324011 synergizes with *CYP2C19**17 to predict a proton pump inhibitor-nonresponsive eosinophilic esophagitis outcome.
- Esophageal eosinophilia patients may benefit from genotype-guided dosing of proton pump inhibitors.

Children treated with proton pump inhibitor (PPI) medications to reduce the inflammation associated with esophageal eosinophilia have initial and sustained response rates of 30% to 60% and 70%, respectively (1,2). The emerging consensus is that

Supplemental digital content is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.jpgn.org).

This work was funded by a grant from the Nemours Foundation; Anonymous Foundation Grant.

The authors report no conflicts of interest.

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DOI: 10.1097/MPG.0000000000002480

PPI medications represent a therapy for eosinophilic esophagitis (EoE) much like dietary elimination and swallowed steroids (3). Whether PPI responsive esophageal eosinophilia (PPI-REE) is, however, mediated by a reduction of esophageal gastric acid exposure or by recently identified anti-inflammatory properties of PPIs, remains controversial (3). Pharmacogenomic factors that influence the outcome of PPI therapy for esophageal eosinophilia remain to be identified.

Individual variability in PPI pharmacokinetics and pharmacodynamics is strongly influenced by genetic variation in *CYP2C19* (4,5). *CYP2C19* variants that confer loss of enzymatic function (LOF, *2, *3, etc) and lead to a poor metabolizer (PM) phenotype, associate with reduced PPI clearance and increased plasma concentrations of PPI compared to normal metabolizers (NM) (6). Similarly, gain of function (GOF, *17) variants have been identified that lead to an extensive metabolizer (EM) phenotype, increased clearance, and decreased plasma concentrations of PPI (6,7). The impact of *CYP2C19**17 GOF allele on PPI-REE in children is not known. In the present study, we hypothesize that carriage of *CYP2C19**17 alleles negatively influences PPI responsiveness in children with EoE. Previous studies have demonstrated that eosinophilic inflammation in EoE is driven by STAT6-dependent local expression of eotaxin-3 (*CCL26*), and that PPIs can block the chromatin remodeling necessary for STAT6 binding and transcriptional activation of *CCL26* (8–13). Therefore, we hypothesize that genetic variants of *STAT6* may also influence responsiveness to PPI treatment in children with EoE.

METHODS

Study Participants

Study participants were prospectively recruited to the parent study at 2 pediatric hospitals in Madrid, Spain between February 2013 and April 2015 as previously described (1). Briefly, children from 2 to 16 years of age who presented with heartburn, chest pain, food impaction, abdominal pain, vomiting, regurgitation, dysphagia, and feeding difficulties, and also had esophageal eosinophilia (≥ 15 eos/0.24 mm², peak value), were enrolled in the primary study. Because *CYP2C19* is not fully expressed in the human liver during infancy (14), only children 2 years or older were included in the present study. Following an initial endoscopy with biopsy, participants were treated with PPI (n = 88 esomeprazole, n = 3 lansoprazole, n = 1 omeprazole; twice daily at a target dose of 1 mg/kg/dose, for a total dose of 2 mg/kg/day, up to a maximum dose of 80 mg/day). The mean duration (standard deviation) of PPI therapy was 10.0 (1.4) weeks, with a high of 13.9 and a low of 4.6 weeks. Three patients who received PPI therapy for <8 weeks were included in the cohort (range 4.6–7.7 weeks). A second endoscopy with biopsy was performed while participants were still taking PPI. As reflected in Figure S1 (Supplemental Digital Content 1, <http://links.lww.com/MPG/B705>), the dose range across all 92 participants was 0.46 to 2.4 mg/kg/day. Three patients received <1 mg/kg/day. The variation in PPI dose (mg/kg/day) in the other 89 patients was either due to reaching the maximum daily dose of 80 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. In the present study, there were a total of 92 patients examined (full cohort), of which 46 were randomly selected to receive pH probe monitoring before initiation of PPI (pH probe cohort) and 46 did not (non-pH probe cohort).

Histological 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 (15,16). All biopsies were targeted to areas with abnormal endoscopic findings if present. Following fixation in 10% buffered formalin and staining with hematoxylin and eosin, eosinophil counts from single high-power microscope fields (hpf) corresponding to an area of 0.24 mm² were recorded. Esophageal eosinophilia was defined as having a peak eosinophil count of ≥ 15 per hpf in 1 or more esophageal biopsy specimens at baseline. After PPI treatment, PPI-REE was defined as <15 eos/hpf and complete PPI-REE was defined as <5 eos/hpf on all esophageal biopsies obtained during the follow-up upper gastrointestinal endoscopy. PPI-nonresponsive EoE was defined as ≥ 15 eos/hpf on any of the esophageal biopsies obtained during the follow-up upper gastrointestinal endoscopy.

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 *STAT6* single-nucleotide polymorphisms (SNPs) interrogated and the TaqMan assays used were rs1059513 (C__7480847_10), rs324015 (C__620398_10), rs3024974 (C__26439023_10), rs841718 (C__7480858_10), rs324011 (C__620399_10), rs167769 (C__620401_20), rs2598483 (C__15984966_10), and rs12368672 (C__31186828_10). The *CYP2C19* SNPs interrogated and assays used were as previously described (17). In this study, no carriers of *CYP2C19* rs4986893 (*3, inactive), rs41291556 (*8, inactive), or rs17884712 (*9, inactive) were identified; therefore, the LOF phenotype that characterizes PMs is defined in this study as carriers of 1 or 2 copies of rs4244285 (diplotypes *1/*2 + *2/*2), without rs12248560 (*17). The GOF phenotype that characterizes EMs is defined as carriers of 1 or 2 copies of rs12248560 (diplotypes *1/*17 + *17/*17) without rs4244285 (*2). Individuals who are *1/*1 are defined as NMs. Diplotypes *2/*17 was not assigned to a metabolizer phenotype.

Statistical Analysis

Analyses were conducted in R base version 3.5.1 (2018) (18). 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. A 2-sided Kolmogorov-Smirnov test was used to test for equality between the empirical distribution functions of 2 samples. Continuous variables were transformed using the *powerTransform* function of the R package *car* (19). Negative binomial regression from the R statistical package *MASS* (20) was employed 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. A Bayesian version of binary logistic regression (function *bayesglm* from package *arm* (21)) was used when perfect separation of a factor was encountered. The Akaike Information Criterion (AIC) (22) was used to assess relative performance of all models. Plots were produced using function *ggplot* from the R statistical package *ggplot2* (23). Probability plots of clinical outcome versus dose of PPI were generated using the *sjp.glm* function from the R statistical package *sjPlot* (24). Linkage disequilibrium between genetic markers was determined using the *r2fast* function of R package *GenABEL* (25). For comparison, LD for the same variants was determined using *rAggr* (26) (<http://raggr.usc.edu/>) within the all European cohort (CEU+FIN+GBR+IBS+TSI) of the 1000 Genomes (27) and HapMap (28) databases. Forest plots were prepared with R package *forestplot* (29). When differences between values

with confidence intervals (CIs) were calculated, the *MOVER-D* method (30) was used to propagate imprecision. Inflation of type-I error through multiple testing has been addressed by correction of reported *P* values using the method of Bonferroni (31).

RESULTS

PPI-REE, GERD, PPI Dose, and CYP2C19

The schema for this study is given in Figure S1 (Supplemental Digital Content 1, <http://links.lww.com/MPG/B705>). The baseline characteristics of study patients in the 5 cohorts examined (pH, non-pH, interquartile range (IQR), non-IQR, and full) stratified by clinical outcome, are given in Table S1 (Supplemental Digital Content 2, <http://links.lww.com/MPG/B706>), of the online supplement. Among the pH probe cohort, 32 (70%) were PPI-REE and 14 (30%) were PPI-nonresponsive EoE. Eight of 32 (25%) patients from the PPI-REE group had an elevated reflux index (>4%) from the baseline pH probe study compared to 3 of 14 (21.4%) patients in the PPI-nonresponsive EoE group (*P* = 1.0). Within the pH probe cohort, the range of PPI doses was 1.18 to 2.33 mg/kg/day. We did not find evidence for an association between reflux index and carriage of *CYP2C19**17 (*P* = 0.35). The probability of achieving a PPI-REE clinical outcome ranged from 41% to 88% when going from 1.18 to over 2.33 mg/kg/day (Fig. S2 Supplemental Digital Content 3, <http://links.lww.com/MPG/B707>). Binary logistic regression modeling (BLRM) of the association between PPI dose and PPI-REE found that for each unit increase in PPI dose, the odds that a patient would have a PPI-REE outcome tended to increase 7.68-fold (PPI-REE odds ratio (OR) [95% confidence interval (CI)] = 7.68 [0.60, 0.97], *P* = 0.11, Fig. S2, Supplemental Digital Content 3, <http://links.lww.com/MPG/B707>).

Next we investigated whether *CYP2C19**17 GOF associates with PPI-REE in the pH probe cohort. BLRM of the association between carriage of *CYP2C19**17 GOF and PPI-REE outcome (dominant genetic model for *CYP2C19**17 GOF with race, sex, age, PPI dose, and PPI type included as covariates) found that children who were carriers of *CYP2C19**17 GOF had 8.2-fold better odds of receiving a PPI-nonresponsive EoE diagnosis than

children who did not carry *CYP2C19**17 GOF (PPI-REE OR [95% CI] = 0.12 [0.02,0.67], *P* = 0.02; complete PPI-REE outcome OR [95% CI] = 0.15[0.03, 0.94], *P* = 0.04; Fig. 1A). Although the pH and non-pH probe cohorts received similar mean (SD) PPI doses (1.79 (1.48) vs 1.83 (1.33) mg/kg/day, *P* = 0.57), larger proportions of patients receive doses at both the low (<1.54 mg/kg/day) and high (>2.05 mg/kg/day) ends of the concentration range in the non-pH probe cohort relative to the pH probe cohort (Fig. 1B). Specifically, a greater fraction of the pH probe cohort fell within the IQR of doses of the full cohort (67% vs 28%, *P* < 0.001, Fig. 1B). Using BLRM (as above), we found that carriage of *CYP2C19**17 GOF was not associated with either PPI-REE or complete PPI-REE outcomes in the non-pH cohort (PPI-REE OR [95% CI] = 1.38 [0.34,5.61], *P* = 0.65; complete PPI-REE OR [95% CI] = 1.54 [0.37,6.46], *P* = 0.56, Fig. 1A). In patients who, however, fell within the IQR of the full cohort for PPI dosage, carriers of *CYP2C19**17 GOF had 7.7-fold better odds of failing PPI therapy and receiving a PPI-nonresponsive EoE diagnosis relative to noncarriers (PPI-REE OR [95% CI] = 0.13[0.02,0.83], *P* = 0.03, Fig. 1A).

STAT6, Baseline Esophageal Eosinophilia, and PPI-REE

We selected eight *STAT6* variants for analysis of associations with outcome of PPI therapy for EoE based on literature reports of associations with EoE (32,33), allergy (34), asthma (35,36), eczema (37), serum immunoglobulin E (IgE) (38,39), or viral infections (40) (Table S2, Supplemental Digital Content 4, <http://links.lww.com/MPG/B708>). Genotype counts, SNP frequencies, and Hardy-Weinberg equilibrium *P* values for the *STAT6* variants are given in Table S2 (Supplemental Digital Content 4, <http://links.lww.com/MPG/B708>). The strongest associations were found when assuming a recessive genetic model. Of the 8 *STAT6* SNPs interrogated, 4 were present at a frequency ≥0.35 in our population, allowing for their analysis using a recessive genetic model (rs841718, rs324011, rs167769, rs12368672; frequency range = 0.36–0.42, Table S2, Supplemental Digital Content 4, <http://links.lww.com/MPG/B708>) (41). Of these 4 SNPs, 3 were in linkage disequilibrium (*r*² ≥ 0.8: rs324011, rs167769, and

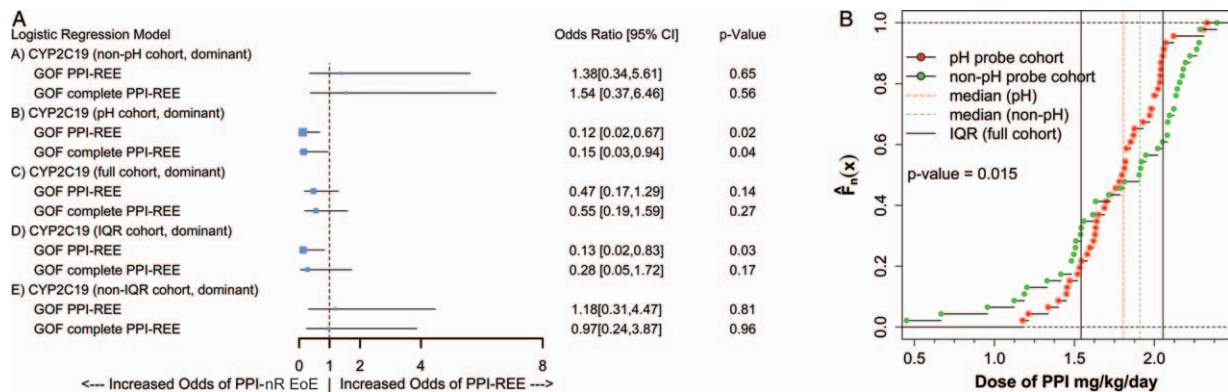


FIGURE 1. Association of *CYP2C19**17 GOF with outcome of PPI therapy is dose dependent. A, Binary logistic regression modeling of *CYP2C19**17 as predictor of PPI therapy outcome: (A) non-pH, (B) pH, (C) full, (D) IQR cohorts, and (E) non-IQR cohorts. All models include race, sex, age, PPI dose, and PPI type as covariates. B, Plot of the empirical cumulative distribution ($\hat{F}_n(x)$) of PPI dose for the pH probe (red) and the non-pH probe (green) cohorts. Solid vertical lines indicate the IQR for the full cohort, dashed vertical lines indicate the medians for the pH probe (red), and the non-pH probe (green) cohorts. The 2 distributions are statistically different from each other (Kolmogorov-Smirnov test *P* value = 0.015). CI = confidence interval; GOF = gain of function; IQR = interquartile range; PPI-REE = proton pump inhibitor-responsive esophageal eosinophilia.

TABLE 1. Association between *STAT6* SNPs and eosinophil counts

SNP	Outcome*	Model	Counts 0/1/2	eos/hpf (0)†	eos/hpf (1)‡	RR‡	P§
rs841718	Distal pre-PPI eos	Recessive	81/11	55[40,67]	80[10,100]	1.21[0.79,1.86]	0.744
rs324011	Distal pre-PPI eos	Recessive	79/13	50[37,64]	85[58,100]	1.56[1.06,2.3]	0.048
rs167769	Distal pre-PPI eos	Recessive	81/11	50[37,64]	100[58,132]	1.66[1.1,2.51]	0.032
rs12368672	Distal pre-PPI eos	Recessive	80/12	50[37,67]	90[58,100]	1.54[1.03,2.31]	0.070
rs12368672	Δ Peak eos (post-pre)	Dominant	33/59	-24.5[-39, -15]	-41.5[-60, -23]	1.58[1.13,2.2]	0.043
rs1059513	Distal post-PPI eos	Additive	72/18/2	10.5[4,17]	1[0,2]	0.31[0.13,0.73]	0.044

eos = eosinophils; RR = rate ratio; pre-PPI = baseline before initiation of proton pump inhibitor; post-PPI = following 8 weeks of PPI therapy; hpf = high power field (0.24mm²).

*Peak value was the highest recorded value from all biopsies in all regions sampled.

†Genetic model coding, recessive: carriage of 0 or 1 copies of the SNP is coded as 0, carriage of 2 copies of the SNP is coded as 1; 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; additive carriage of 0 copies of the SNP is coded as 0, carriage of 1 copy of the SNP is coded as 1, carriage of 2 copies of the SNP is coded as 2.

‡Median (95% CI) are reported.

§Reported value is from negative binomial regression modeling with eosinophil counts as the dependent variable and genotype counts as the independent variable. Values in bold indicate associations that are significant. A Bonferroni correction has been applied to P values where appropriate.

rs12368672; Fig. S3, Supplemental Digital Content 5, <http://links.lww.com/MPG/B709>.

In our initial analysis (1), we determined that pre-PPI eos/hpf tended to be higher in patients who would eventually fail PPI therapy (peak eos/hpf median [95% CI], PPI-REE = 45[32,65] vs PPI-nonresponsive EoE = 83[71,100], P = <0.01, Table S1, Supplemental Digital Content 2, <http://links.lww.com/MPG/B705>). Therefore, we examined the association between *STAT6* variants and pre-PPI eos/hpf in the full cohort of 92 patients (Table 1). Carriers of 2 copies of any of the 4 SNPs (rs841718, rs324011, rs167769, rs12368672) tended to have elevated distal pre-PPI eos/hpf relative to individuals who had 0 or 1 copy (range of the median difference in distal eos/hpf [95% CI], = 25[-46,50] to 50[6,85], Table 1). In particular, 2 SNPs were associated with a >1.7-fold increase in distal pre-PPI eos/hpf in individuals who carry 2 copies relative to individuals who carry 0 or 1 copy: rs324011 (PPI-REE rate ratio (RR) [95% CI] = 1.56 [1.06, 2.3], P = 0.048) and rs167769 (PPI-REE RR [95% CI] = 1.66 [1.1,2.51], P = 0.032).

Next, we tested for associations between the change in post-PPI versus pre-PPI eos/hpf (Δ peak eos/hpf) in individuals who carried 1 or 2 copies of any of the 8 interrogated *STAT6* variants (Table 1). We found that carriers of 1 or 2 copies of rs12368672

(frequency = 0.39, Table S2, Supplemental Digital Content 4, <http://links.lww.com/MPG/B708>) have a Δ peak eos/hpf that is 1.7-fold larger than that seen in individuals who do not carry rs12368672 (median difference eos/hpf [95% CI], = -17 [-38,7], RR [95% CI] = 1.58 [1.13, 2.2], P = 0.043, Table 1). Carriers of rs1059513 (frequency = 0.12, Table S2, Supplemental Digital Content 4, <http://links.lww.com/MPG/B708>) have a 10.5-fold lower post-PPI eos/hpf relative to noncarriers (median difference eos/hpf [95% CI], = -9.5 [-16, -3], RR [95% CI] = 0.31 [0.13, 0.73], P = 0.044, Table 1).

Finally, we tested for associations between carriage of *STAT6* variants and outcome of PPI therapy (Fig. 2). Given the previous results, we focused our analysis on three of the four linked variants rs324011, rs167769, rs12368672, and on rs1059513. In BLRMs examining the association between *STAT6* variant and a PPI-REE outcome (dominant genetic model, covariates as above), individuals who carried 1 or 2 copies of rs1059513 had 6.2-fold better odds of achieving a PPI-REE outcome after PPI therapy than individuals who did not carry rs1059513 (PPI-REE OR [95% CI] = 6.16 [1.44, 26.35], P = 0.028) (Fig. 2). When considering complete PPI-REE, the odds improved to 7-fold more likely for carriers of rs1059513 relative to noncarriers (PPI-REE OR [95% CI] = 7.06 [1.98, 24.9], P < 0.01).

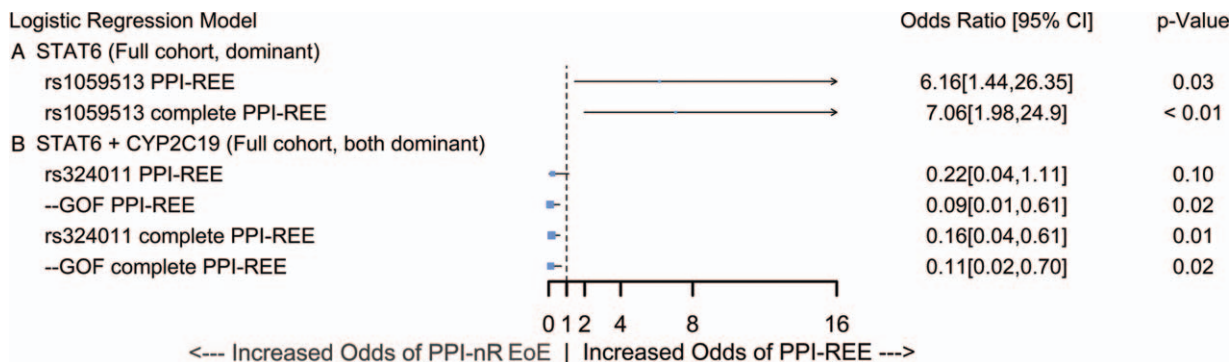


FIGURE 2. PPI-REE outcome in carriers of *STAT6* and *CYP2C19**17 GOF alleles. Binary logistic regression modeling of: (A) *STAT6* rs1059513 as predictor of PPI-REE/complete PPI-REE outcome following 8 weeks of PPI therapy in the full cohort and (B) *STAT6* and *CYP2C19**17 GOF as co-predictors of PPI-REE in the full cohort. All models include race, sex, age, PPI dose, and PPI type as covariates. CI = confidence interval; GOF = gain of function; PPI-REE = proton pump inhibitor-responsive esophageal eosinophilia.

CYP2C19, STAT6, and PPI-REE

Terms for *STAT6* (dominant genetic model), *CYP2C19* (dominant genetic model), and their interaction were included in BLRMs with PPI-REE or complete PPI-REE as the outcome (covariates as above, Fig. 2). We found that when rs1059513 and *CYP2C19**17 GOF were modeled as copredictors of outcome, carriers of 1 or 2 copies of rs1059513 had 14.9-fold better odds of responding to PPI therapy and receiving a diagnosis of PPI-REE than individuals who did not carry rs1059513—when considering only individuals who do not carry *CYP2C19**17 GOF (PPI-REE OR [95% CI] = 14.9 [1.87, 119], $P = 0.02$; complete PPI-REE OR [95% CI] = 7.02 [1.88, 26.3], $P < 0.01$). When rs324011 and *CYP2C19**17 GOF were modeled as co-predictors of outcome, rs324011 did not significantly predict PPI-REE but did significantly predict complete PPI-REE. Carriers of 1 or 2 copies of rs324011 had 6.10-fold better odds of failing to achieve complete PPI-REE relative to non-carriers (PPI-REE OR [95% CI] = 0.22 [0.04, 1.11], $P = 0.10$; complete PPI-REE OR [95% CI] = 0.16 [0.04, 0.61], $P = 0.01$). While the model for rs1059513 did not benefit from the addition of the *CYP2C19* GOF term as shown by a positive Δ AIC: 2.11 and a nonsignificant P value for the *CYP2C19**17 GOF term, the model for rs324011 did (Δ AIC complete PPI-REE: -2.94 ; PPI-REE OR [95% CI] = 0.11 [0.02, 0.70], $P = 0.02$). The OR of the interaction term between rs324011 and GOF (PPI-REE OR [95% CI] = 8.76 [0.90, 84.8], $P = 0.05$) implies that the influence of *CYP2C19**17 GOF allele on EoE outcome increases 8.76-fold as rs324011 increases from 0 copies to 1 or 2 copies.

DISCUSSION

The present study reports novel associations between common genetic variants of *CYP2C19* and *STAT6* and PPI-REE in children who received high-dose PPI therapy for pediatric esophageal eosinophilia. Previously, we have shown that carriage of *CYP2C19**17 GOF alleles associates with pH probe acid exposure outcomes (17) in children with gastroesophageal reflux disease. In the present study, clinically significant esophageal acid exposure, however, does not differentiate PPI-REE from PPI-nonresponsive EoE, and we did not find an association between *CYP2C19**17 and pH probe acid exposure outcomes. We did demonstrate that carriers of *CYP2C19**17 GOF have 8.2-fold better odds of failing PPI therapy and receiving a PPI-nonresponsive EoE diagnosis than children who did not carry *CYP2C19**17 GOF (PPI-REE OR [95% CI] = 0.12 [0.02, 0.67], $P = 0.02$) in a cohort of patients who received pH probe monitoring. Although patients were randomly chosen to receive pH probe monitoring, a classification tree analysis found that most individuals within the pH probe cohort received a PPI dose within the range of ≥ 1.569 to < 2.075 mg/kg/day, which corresponds well with the IQR dose range of ≥ 1.54 to < 2.05 mg/kg/day for the full cohort. Subsequent analysis of the IQR cohort confirmed that carriers of *CYP2C19**17 GOF have 7.7-fold better odds of failing PPI therapy and receiving a PPI-nonresponsive EoE diagnosis relative to noncarriers (PPI-REE OR [95% CI] = 0.13 [0.02, 0.83], $P = 0.03$). These results suggest that carriage of *CYP2C19**17 GOF may only influence outcome of PPI therapy within a range of PPI doses (including > 1.54 and < 2.05 mg/kg/day).

In the present study, we show that 2 linked variants of *STAT6*, rs324011, and rs167769 associate with increased pre-PPI eos/hpf and that carriage rs324011 also predicts failure of PPI therapy in binary logistic regression models that include *CYP2C19**17 GOF variant as a copredictor. Remarkably, we find a significant interaction between *CYP2C19**17 GOF and *STAT6* variant rs324011

suggesting that the influence of *CYP2C19**17 GOF on outcome of PPI therapy for EoE increases almost 9-fold in individuals who are carriers of rs324011. We also find that individuals who carry 1 or 2 copies of rs1059513 have 6.2-fold better odds of achieving a PPI-REE outcome following PPI therapy than individuals who did not carry rs1059513 (PPI-REE OR [95% CI] = 6.16 [1.44, 26.35], $P = 0.02$). In subanalyses we found that these results were robust for the entire intention-to-treat population, which included 4 patients who received PPIs other than esomeprazole, 3 patients who received PPI therapy for < 8 weeks, and 5 patients who received ≤ 1 mg/kg/day PPI (Supplementary Analysis S1 Supplemental Digital Content 6, <http://links.lww.com/MPG/B710>).

One proposed mechanism for the effect of PPI on EoE is reduced esophageal exposure to gastric acid as is seen with gastroesophageal reflux disease. A recent systematic review and meta-analysis of PPI trials for EoE, however, failed to demonstrate a significant trend in response between patients with pathologic versus normal pH probe outcomes (65% vs 45%) (42), which is consistent with our findings in the present study. The effects of *CYP2C19**17 allele appears to be exerted within a specific range of PPI doses, empirically defined in this study by the IQR of the PPI dose range and does not appear to exert influence at the low and high ends of the dose range. This finding is consistent with the possibility that the high PPI dose range employed by this study compensates for carriage of *CYP2C19**17. This general strategy of therapeutic dose adjustment to compensate for variants of drug metabolizing enzymes is the cornerstone of precision medicine (43).

Several studies have shown that PPIs block *STAT6* binding to and transcriptional activation of *CCL26* (12,13), which is an important chemokine that mediates chemotaxis of eosinophils to the esophagus in EoE (44,45). Variants of *STAT6* are known to be associated with diseases that are driven by allergic inflammation including allergy (34), asthma (35,36), eczema (37), serum IgE (38,39), or viral infections (40) and food allergies (46), and a recent genome wide association study conducted by Rothenberg et al (32) identified a variant of *STAT6* (rs167769) that is strongly associated with EoE. Upon activation of ST2 expressing cells by IL-33, production of IL-13 is increased (47) leading to activation of *STAT6* via IL-4R (48). *STAT6* upregulates GATA-3, the master regulator of Th2 inflammatory cell differentiation, IgE class switching in B cells, and expression of major histocompatibility complex class II and CD23 (a low-affinity receptor for IgE (Fc ϵ R2)), thus increasing antigen presentation and immune reactivity (49–52). Specifically, *STAT6* upregulates transcription of *CCL26* (eotaxin-3) 53-fold in esophageal eosinophilia relative to levels found in peptic esophagitis (9) and 490-fold over levels found in normal esophageal biopsies (11). We confirm that common genetic variants of *STAT6* influence response to PPI therapy for EoE.

This study had several limitations including small sample size, variation in PPI dose and length of therapy, lack of pH measurement in a large portion of the cohort, lack of a validated questionnaire for symptom assessment, and the potential for additional genetic variants identified in previous genome wide association study studies (32,33) to act as confounders and influence clinical outcome of PPI therapy.

CONCLUSIONS

In conclusion, the effect of PPI medications in pediatric EoE appears to be through a dose-dependent mechanism associated with *CYP2C19**17 that does not correlate with esophageal gastric acid

exposure as measured by pH probe monitoring. Genetic variants of *STAT6* associate with pre-PPI eos/hpf (rs324011, rs167769, and rs12368672), PPI-REE (rs1059513), and interact with *CYP2C19**17 to increase the odds of PPI-nonresponsive EoE (rs324011). Taken together, our results suggest that genetic variants in *CYP2C19* and *STAT6* are important factors that influence the pharmacogenetics/genomics of PPI therapy in EoE. Furthermore, our data support an anti-inflammatory mechanism for PPI efficacy in EoE. Pediatric EoE patients may benefit from future genotype-guided personalized PPI therapy.

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