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Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids

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Abstract

Background—Esophageal remodeling occurs in eosinophilic esophagitis (EE) patients but whether the components of remodeling in the subepithelium are reversible by administration of topical oral corticosteroids is unknown.

Methods—We quantitated the degree of lamina propria remodeling in esophageal biopsies obtained before and after at least 3 months of therapy with budesonide in 16 pediatric EE subjects. In addition, we investigated whether corticosteroid therapy modulated vascular activation (expression of VCAM-1; level of interstitial edema), TGF β ₁ activation (levels of TGF β ₁, phosphorylated Smad2/3), and performed a pilot analysis of a polymorphism in the TGF β ₁ promoter in relation to EE subjects who had reduced remodeling with budesonide therapy.

Results—EE subjects were stratified based on the presence ($n = 9$) or absence ($n = 7$) of decreased epithelial eosinophilia following budesonide. Patients with residual eosinophil counts of ≤ 7 eosinophils per high power field in the epithelial space (responders) demonstrated significantly reduced esophageal remodeling with decreased fibrosis, TGF β ₁ and pSmad2/3 positive cells, and decreased vascular activation in association with budesonide therapy. Responders were more likely to have a CC genotype at the -509 position in the TGF β ₁ promoter.

Conclusions—Reductions in epithelial eosinophils following budesonide therapy were associated with significantly reduced esophageal remodeling.

Keywords

budesonide; eosinophilic esophagitis; pediatric; remodeling; transforming growth factor-beta (TGF β)

Eosinophilic esophagitis (EE) is a disease of increasing prevalence and is diagnosed if esophageal histology demonstrates >15 epithelial eosinophils per high power field (hpf), especially in the context of acid blockade to exclude gastroesophageal reflux (GERD) (1–3).

Patients may present with dysphagia, vomiting, abdominal pain, or poor growth and the most significant complication is esophageal stricture formation requiring repeated dilation (1,4,7).

Subepithelial fibrosis has been described in adult and pediatric EE patients, especially those who complain of dysphagia (5–8). Our previous studies demonstrated that significant esophageal remodeling occurs in the lamina propria (LP) of the esophagus of pediatric EE patients with concurrent strictures as compared with normal controls or children with GERD (5). Subepithelial fibrosis is accompanied by increased numbers of TGF β -positive eosinophils and increased expression of the TGF β signaling transcription factor, phosphorylated Smad2/3 (pSmad2/3) (5,6). In addition, EE is associated with an increased number of activated blood vessels expressing VCAM-1 in the subepithelial space (5). Whether LP remodeling changes are influenced by therapies such as topical esophageal corticosteroids has not been systematically assessed in EE patients.

Currently, EE studies utilize epithelial eosinophil resolution as the major diagnostic criterion for therapeutic response. The previous studies of topical corticosteroids such as fluticasone and budesonide in pediatric EE have demonstrated a 50–80% overall resolution of epithelial inflammation and clinical symptoms (1,9–11). However, a difference in the resolution of esophageal remodeling among patients who do and do not respond to corticosteroid therapy has not been reported. We therefore hypothesized that EE subjects who demonstrated resolution of epithelial eosinophilia following topical corticosteroid therapy would be more likely to have resolution of LP remodeling than those who did not demonstrate resolution of epithelial eosinophilic inflammation. In addition, as LP is not obtained in all esophageal biopsy samples, it is important to determine whether changes in the epithelium (obtained in all esophageal biopsies) correlates with changes in LP fibrosis and inflammation. As current EE therapy involves repeated esophagogastroduodenoscopy (EGD) with biopsy, the relationship between esophageal remodeling and clinical features and disease course, even in young children, can be determined.

In this study, we demonstrate that treatment with swallowed topical budesonide decreases the severity of esophageal remodeling (LP fibrosis and vascular activation) and mediators (TGF β ₁ and pSmad2/3) in EE patients who demonstrate resolution of epithelial eosinophilia but not in those who do not have an epithelial response to corticosteroid therapy. In a pilot study, we demonstrate that a SNP in the TGF β promoter may correlate with patients who respond to therapy.

Materials and methods

EE subjects

A database of EE patients who have been evaluated in the Eosinophilic Gastrointestinal Disorders (EGID) clinic at Rady Children's Hospital, San Diego (RCHSD) from 1998 forward was searched and identified 16 EE patients from 2004–2006 who met the following inclusion/exclusion criteria:

1. Therapy with swallowed budesonide (Pulmicort®; AstraZeneca LP, Wilimington, DE, USA (product of Sweden)) 1 mg daily mixed with sucralose, dextrose, and maltodextrin (Splenda®; McNeil Nutritionals, LLC, Ft. Washington, PA, USA) for at least 3 months during routine clinical care
2. Pre- and post-therapy specimens with adequate LP for analysis
3. Histologic evidence of EE defined as a peak esophageal eosinophil count of ≥ 20 per hpf at 400 $\times$ light microscopy prior to budesonide therapy (Table 1)

4. No change in diet, environment, and medications between the baseline and follow-up biopsies
5. Meet criteria for 'responder' or 'non-responders' defined as patients whose post-therapy biopsy had maximal epithelial eosinophil counts of ≤ 7 per hpf and ≥ 20 eosinophils per hpf, respectively. Patients with intermediate response (7–20 eosinophils per hpf) were excluded from the analysis (only one patient from the 2004–2006 database review was excluded)

All histologic and immunologic stains were analyzed by an investigator blinded to the patient biopsy identity and response to therapy. All assessments were made using an Olympus BH-2 light microscope. The size of a hpf with a 40 $\times$ objective is 0.17 mm². Institutional review board approval for the study was granted by RCHSD and the University of California, San Diego.

Symptoms were scored using chart review (RD, SA). Scores of 0 = no symptoms, 1 = mild symptoms/intermittent lifestyle interference, 2 = severe symptoms/daily lifestyle interference were assigned to heartburn/regurgitation, nausea/vomiting, dysphagia/odynophagia, anorexia/early satiety, nocturnal awakening, and abdominal pain.

Quantitation of epithelial features

The peak number of eosinophils was counted in five high power fields (hpf) at 400 $\times$ light microscopy using a hematoxylin and eosin stain. The mean eosinophil count per hpf was calculated by averaging the eosinophil counts in 6 hpf at two esophageal levels. Epithelial desquamation was graded as present = 1 or absent = 0. Basal zone hyperplasia (BZH) was scored as the percent of the total epithelial height that was occupied by the basal zone in well oriented specimens. Normal patients have <20% basal zone and this was scored as 0. BZH was graded as 1 to 3: 1 = 21–50%, 2 = 51–75%, 3 = >75% of the total epithelial thickness. A composite epithelial score was generated by adding each score for these components.

Epithelial intercellular edema was assessed as widened intercellular spaces (14) and was scored as 0 = absent or 1 = present.

Quantitation of esophageal remodeling in the LP eosinophils and fibrosis

The peak number of eosinophils in 3–5 hpf was quantitated in the LP at the site of remodeling. The severity of LP fibrosis was assessed using a hematoxylin and eosin stain in a region from 70 to 150 microns immediately below the epithelium and was scored from 0 to 3 by evaluation of the amount and thickness of collagen bundles and number of fibroblasts as previously described (5).

Detection of TGF β ₁ and pSMAD2/3 in EE

Esophageal biopsies were immunostained with primary antibodies to TGF β ₁ or pSmad2/3 (both from Santa Cruz Biochemicals, Santa Cruz, CA, USA) as previously described (5). Cells were enumerated in three to five separate fields. Results are expressed as the number of TGF β or pSmad2/3 positive cells/hpf at 400 $\times$ light microscopy.

Vascular changes in EE

The number of VCAM-1 (R&D, Minneapolis, MN, USA) positive blood vessels in the LP was quantitated at 400 $\times$ fluorescence microscopy in three to five separate fields as previously described (5). Results are expressed as the number of VCAM-1 positive blood vessels/hpf.

Analysis of the C-509T SNP of the TGF β 1 promoter

Analysis of the C-509T SNP of the TGF β 1 promoter was performed using DNA isolated from peripheral blood samples of EE patients. The TGF β promoter was sequenced using forward (CAGACTCTAGAGACTGTCAG) and reverse (GTCACCAGAGAAAGAGGAC) primers for PCR and direct sequencing for C-509T SNP genotype. Sequence analysis was performed using Sequencher (Genecodes, Ann Arbor, MI, USA). A separate written informed consent approved by the RCHSD and UCSD IRB was obtained for genotyping. To increase the numbers of genotyped patients, we utilized 12 (eight responders, four non-responders) of the 16 EE patients described in this report (four were unable to be consented) and an additional eight EE subjects who met all entry criteria for this study (five responders and three non-responders) but whose paired esophageal biopsies did not have sufficient LP for remodeling analysis. All of the additional patients were similar in age, histologic, endoscopic, and symptom findings ($P \geq 0.05$, Table 1, data not shown).

Statistical analysis

Statistical analysis was performed utilizing the NCSS statistical package. Pre- and post-therapy features were compared using a Wilcoxon test, comparisons between groups were made using Mann–Whitney test. Correlation coefficients (Spearman r) were calculated using GRAPHPAD PRISM software. Fisher's exact test was generated using a 2×2 table. A two-tailed P value of <0.05 was considered statistically significant.

Results

Clinical features

Nine responders (defined as patients who had ≤ 7 epithelial eosinophils per hpf following budesonide therapy) and seven non-responders (defined as having a residual epithelial eosinophilia of >20 per hpf) met study criteria. Patients in both groups had similar ages, atopic status, budesonide duration, and clinical features (Table 1). With the exception of dysphagia, which was more common among the non-responders, the patients in each group had similar symptom complaints. There was a statistically nonsignificant trend for disease duration (defined as the time of the first biopsy meeting criteria for EE to the onset of budesonide therapy) to be shorter in the responder patients.

Effect of budesonide on the esophageal epithelium and epithelial eosinophils

We have previously reported that patients treated with topical budesonide have healing of the esophageal epithelium (10). The current patient population was selected to study the relationship between the epithelial and LP remodeling response.

The mean peak eosinophil count in the esophageal epithelium among responder and non-responder EE subjects was not different prior to therapy (Table 1). Following budesonide therapy, the mean peak epithelial eosinophil count remained significantly elevated in non-responders (mean = 59, median = 56 per hpf, 95% CI 42, 75) when compared with responders (mean = 2.5, median = 1, 95% CI 0.15, 6) (Table 1, Fig. 1A, C–F). An analysis of the data using mean eosinophils per hpf showed mean eosinophil counts of 43 (responders, 95% CI 28, 57) and 42 per hpf (non-responders, 95% CI 25, 60) before therapy. Following therapy, mean eosinophil counts were 0.6 (95% CI 0.03, 1.2) and 43 per hpf (95% CI 24, 62) in responders and non-responders, respectively.

To evaluate changes in the epithelial features among responders and non-responders, we utilized a pathology score that includes the severity of BZH and epithelial desquamation. Both groups were comparable prior to therapy with scores of 2.2 (95% CI 1.2, 3.2, median = 2) and 2.5 (95% CI 1.1, 3.9, median = 3) among responders and non-responders, respectively.

Following therapy, only the responders had a significant decrease in epithelial score to 0.2 ($P = 0.007$), while non-responders had post-therapy scores of 2.3 (Fig. 1B).

Esophageal LP remodeling in responder vs non-responder patients LP eosinophils

As subepithelial eosinophils are a source of $\text{TGF}\beta_1$ and required for remodeling (5,6), we examined whether eosinophil numbers in the LP followed a similar response pattern to epithelial eosinophils. Responders had significantly decreased eosinophils per hpf, 12.5 per hpf (95% CL 2.8, 22; median = 7) prior to and 1.8 per hpf following budesonide therapy (95% CL 0.5, 3; median = 1) ($P = 0.02$) (Fig. 2A). In non-responders, mean peak eosinophil count increased from 13 per hpf (95% CL 4, 21; median = 11) before to 32 eosinophils per hpf (95% CL 14, 50; median = 23) following budesonide therapy (Fig. 2A).

Fibrosis

In responders, the LP fibrosis score decreased significantly from 1.6 before therapy (95% CL 0.8, 2.3; median = 1) to 0.67 post-therapy (95% CL 0.12, 1.2; median = 1) ($P = 0.001$) (Figs 1C–H and 2B). Non-responders had unchanged fibrosis scores following therapy (mean = 2.3, median = 2.5 95% CL 1.3, 3.3 prior to therapy; mean = 2.4, median = 2, 95% CI 1.9, 2.9 post-therapy) (Fig. 2B).

Lamina propria $\text{TGF}\beta_1$, pSmad2/3 in responders vs non-responders

As $\text{TGF}\beta_1$ and its signaling pathway (pSmad2/3) can be important mediators of fibrosis, we examined the number of cells expressing these molecules among responder and non-responders. Prior to budesonide therapy the mean number of $\text{TGF}\beta_1$ positive cells/hpf in the LP did not differ significantly between responders (mean = 84 $\text{TGF}\beta_1$ positive cells/hpf, 95% CL 46, 123; median = 91) and non-responders (mean = 109, median = 108 95% CL 71, 148) (Fig. 2C). Following budesonide, only responders had significantly decreased numbers of $\text{TGF}\beta_1$ positive cells/hpf (mean = 35, median = 18, 95% CL 8, 54; median = 18) ($P = 0.01$) (Fig. 2C, E). Non-responders continued to have elevated numbers of $\text{TGF}\beta_1$ positive cells/hpf post-therapy (mean = 97, median = 100, 95% CL 69, 125) ($P = 0.005$).

To evaluate the effects of budesonide on the pathway downstream from $\text{TGF}\beta_1$, we analyzed the number of pSmad2/3 positive cells before and after budesonide therapy. Prior to budesonide therapy, both groups had a similar pSmad2/3 positive cells (mean = 152, 95% CI 121, 182 in responders vs mean = 125, 95% CI 94, 156 in non-responders, $P = 0.15$) (Fig. 2D). Following budesonide therapy, only responders had a significant decrease in the mean number of pSmad2/3 positive cells/hpf (mean = 86, 95% CL 56, 115; $P = 0.008$), whereas non-responders continued to have elevated numbers of pSmad2/3 positive cells (mean = 119 per hpf; 95% CL 61, 178) (Fig. 2D, F).

VCAM-1 and interstitial edema in responders vs non-responders

We have previously demonstrated that there is increased vascular activation in the LP of strictured EE patients when compared with GERD or normal patients (5). Responders demonstrated a decrease ($P = 0.04$) in the number of VCAM-1 positive vessels with 19 VCAM-1 positive vessels per hpf (95% CL 11, 26; median = 15) prior to, and 13 VCAM-1 positive vessels/hpf (95% CL 7, 18; median = 11) following budesonide therapy (Fig. 3A). Non-responders had similar mean numbers of VCAM-1 positive blood vessel counts before and after budesonide therapy with 21 (95% CI 11, 26) prior to and 20 VCAM-1 positive vessels per hpf (95% CL 13, 28; median = 20) following budesonide therapy (Fig. 3A).

The histologic finding of epithelial edema, in the form of dilated intercellular spaces, may be a reflection of vascular permeability (14). Responders had a statistically significant decrease

in intercellular edema (0.55 prior to therapy, 0 following therapy, $P = 0.03$) when compared with non-responders who had no change in edema following budesonide therapy (0.71 prior to therapy, 0.7 following therapy Fig. 3B).

Eosinophil numbers and TGF β_1 promoter genotypes (CC, CT, TT)

Fibrosis and disease severity can be related to functional promoter SNPs in the TGF β_1 gene (13). To perform a pilot study of the TGF β_1 C-509T SNP that is associated with severe asthma (13,15,17) in an EE population, we analyzed the frequency of the CC, CT, TT alleles in 20 EE patients. In EE patients, the CC genotype was present in 30% of EE subjects (6/20), the CT genotype in 60 % of EE subjects (12/20), and the TT genotype in 10% of EE subjects (2/20) (Tables 1 and 2). The frequency of these alleles in EE did not differ from that found in a general Caucasian population (15). However, the CC genotype correlated with responder status in the complete genotyped cohort ($n = 20$, $P = 0.04$) and approached statistical significance in the original cohort available for genotyping ($n = 12$, $P = 0.07$). The likelihood ratios for CC genotype and response were 4 ($P = 0.04$) in the original cohort and 5.7 ($P = 0.02$) in the total genotyped cohort. As the CC genotype lacks the creation of a YY-1 binding site and thus, theoretically lowers TGF β_1 expression, we compared the numbers of TGF β_1 positive cells in the LP by genotype. EE patients with a CC genotype had significantly lower numbers of TGF β_1 positive cells than their non-CC (i.e. CT or TT) counterparts prior to therapy ($P = 0.01$ using the original or complete genetics cohort) (Fig. 4).

Discussion

Our data demonstrate the novel finding that epithelial EE improvement following topical budesonide is associated with improvements in LP remodeling. We demonstrate that responder patients with decreased epithelial eosinophil counts had significant reductions in subepithelial fibrosis, an important feature of esophageal remodeling. The significant reduction in the numbers of TGF β_1 and pSmad2/3 positive cells in responders suggests one potential mechanism by which budesonide may impact esophageal remodeling. Responders also had reduced VCAM-1 positive vessels and decreased epithelial interstitial edema in association with epithelial budesonide response. We chose to use peak eosinophil counts to be consistent with current consensus guidelines and our previous studies (1,5,10).

In our study, the strongest predictor of reduced remodeling was evidence of significantly reduced epithelial eosinophils in esophageal biopsies following budesonide. Indeed eosinophil numbers in the epithelium and LP were significantly correlated with one another and to the degree of fibrosis and numbers of TGF β_1 and pSmad2/3 positive cells ($r = 0.5-0.6$, $P \leq 0.01$). As eosinophils produce pro-fibrotic factors such as TGF β_1 , are increased in the LP of pediatric EE patients, and are required for esophageal collagen deposition in animal models (4-6), the effects of therapies that reduce eosinophil numbers is likely an important mechanism of decreased remodeling in responder patients. In addition, corticosteroids may reduce TGF β_1 mediated fibrosis by decreasing signaling through the IL-13-AP-1 pathway, such as in asthma (12,16).

As not all patients have decreased inflammation following corticosteroid therapy and as patients with persistent epithelial inflammation had persistent fibrosis, we were interested in investigating whether genetic polymorphisms in the TGF β_1 promoter (CC, CT, TT) might associate with disease resolution. Interestingly, all of the patients with a TGF β_1 promoter CC genotype in this pilot study had significant reductions in esophageal remodeling following budesonide therapy. As the TGF β_1 promoter TT genotype is associated with higher serum TGF β_1 levels in asthmatic patients (15,17) and with increased airway obstruction despite corticosteroid therapy in severe asthma, it is intriguing to hypothesize that patients with a TGF β_1 promoter CC genotype have a less fibrotic form of EE. The TT genotype produces a

transcription binding site for YY-1, increases promoter activity, and may predispose patients to increased fibrosis, rendering them less responsive to corticosteroids. The fact that the TGF β ₁ promoter CC allele EE patients had significantly lower numbers of subepithelial TGF β ₁ positive cells and that all are corticosteroid responsive is consistent with this hypothesis. An improved understanding of the pharmacogenomic characteristics of EE patients will be important in the future management of pediatric EE patients. This small pilot analysis of TGF β ₁ promoter and the suggestion that C-509T could have prognostic potential for disease resolution in response to therapy is intriguing and merits further study in larger EE population based studies that are adequately powered to address this question.

Our data also demonstrate that young children without strictures can have esophageal remodeling. The patients reported in this study had significantly increased levels of eosinophils, fibrosis, and vascular activation when compared with the normal children (data not shown). Although all our EE study subjects reported using their budesonide therapy, we are unable to definitively determine from this study whether nonadherence to budesonide therapy, vs resistance to budesonide, explains the lack of response in a subset of EE subjects. As our study is retrospective and lacks a placebo control, ongoing prospective placebo controlled trials with budesonide will help to demonstrate if remodeling may resolve spontaneously in certain patients. Although our current study lacks a placebo group, we analyzed remodeling features in patients ($n = 4$) who were noncompliant with the prescribed therapy (mean = 7.5 months). Prior to therapy this group did not differ in age, epithelial eosinophil counts, TGF β ₁, pSmad2/3 counts, or fibrosis score from our described cohort. Follow-up biopsies showed persistence of all these parameters suggesting that, without an intervention, remodeling features are unlikely to improve 7 months (data not shown).

In summary, epithelial eosinophil reductions in the esophagus correlate with a concomitant reduction in LP eosinophils, fibrosis, numbers of TGF β ₁ and pSMAD 2/3 positive cells, and vascular activation in the esophagus following budesonide therapy. As adequate LP for study of esophageal remodeling is not available in all routine clinical biopsy specimens, it is of interest that our data demonstrates that those patients with an epithelial response to therapy will also have a deeper LP response. Further studies are needed in patients with EE and strictures to determine whether features of remodeling can be reduced by corticosteroids in such patients. Alternatively, if the phenotype/genotype of EE patients at risk for developing extensive remodeling and strictures can be identified, early intervention with budesonide and/or other therapies may prevent stricture formation. Lastly, the fact that young children with EE undergo repeated EGD with biopsy over prolonged time periods makes EE a new and useful model system for studying human eosinophil-associated tissue remodeling that occurs early in life.

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Abbreviations

EE	eosinophilic esophagitis
GERD	gastroesophageal reflux disease
BZH	basal zone hyperplasia
TGF β	transforming growth factor beta-1

pSmad2/3	phosphorylated Smad2/3
Hpf	high power field
VCAM-1	vascular cell adhesion molecule-1
SNP	single nucleotide polymorphism

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Figure 1.

Epithelial eosinophil and epithelial changes in EE patients treated with budesonide. The peak epithelial eosinophil count prior to (Pre) and following (Post) therapy among responders (RESP, ≤ 7 eos/hpf) and non-responders is shown (NON-RESP, >20 eos/hpf) (A). Epithelial scores grade the severity of basal zone hyperplasia and presence of epithelial desquamation among responders (RESP, ≤ 7 eos/hpf) vs non-responders (NON-RESP, >20 eos/hpf) prior to (Pre) and following (Post) budesonide therapy (B). Bars represent means. Representative images of hematoxylin/eosin stained biopsy in responder patient shows epithelial basal zone hyperplasia, dilated intercellular spaces, eosinophils and degranulation (C, E), lamina propria fibrosis with eosinophils prior to therapy (C, G) and resolution following therapy (D, H, F).

Figure 2.

Lamina propria fibrosis and pro-fibrotic mediators in EE patients in response to budesonide therapy. Lamina propria eosinophils (A), fibrosis scores (B), TGF β ₁ (C) and phosphorylated Smad2/3 (pSmad2/3) expressing cells (D) in the lamina propria (LP) of EE patients who have decreased (RESP, $\leq 7\text{eos/hpf}$) and continued (NON-RESP, $>20\text{eos/hpf}$) epithelial eosinophils prior to (Pre) and following (Post) therapy. Bars represent means. Representative images of TGF β ₁ positive (brown) (E) and pSmad2/3 (F) positive (red) cells prior to (left panels) and following therapy (right panels) in a responder patient.

Figure 3.

Lamina propria (LP) vascular activation and dilated intercellular spaces among responders and non-responders. LP VCAM-1 positive vessels (A) and epithelial dilated intercellular spaces (B) in patients who have decreased (RESP, ≤ 7 eos/hpf) and continued (NON-RESP, >20 eos/hpf) epithelial eosinophils prior to (Pre) and following (Post) therapy. Bars represent means.

Figure 4.

TGFβ₁ cell numbers are lower in patients with CC promoter genotype. Numbers of lamina propria TGFβ₁ positive cells in patients with -509 CC or CT/TT genotype in the TGFβ₁ promoter. R = Responders, patients with CT or TT genotype who had ≤ 7eos/hpf following budesonide therapy. Bars represent means.

Table 1

Clinical and histologic features of patients.* Responders have ≤ 7 eosinophils per high power field (hpf) following therapy; Non-responders have >20 eosinophils per hpf. † Additional genetics patients

Patients	Age (year, month)	Gender	Atopy [†]	Genotype	Medications	Peak Eos/HPF	
						Pre	Post
Responders*							
1	2, 10	M	None	CC	PPI	100	0
2	17, 9	F	AR	CT	PPI, Levettiracetam, Oxcarbazepine	130	0
3	3, 5	M	AR	CC	PPI	55	0
4	1, 1	M	FA	CC	PPI	52	0
5	1, 5	M	FS	TT	PPI	54	1
6	7, 6	F	FS, IA	CT	CoQ10, Insulin, DDAVP	170	3
7	2, 8	M	Eczema, AR, FA, PA	ND	PPI	100	5
8	3, 9	M	None	CT	PPI	30	7
9	4, 3	M	FS, AR	CC	None	20	7
10 [†]	4, 3	F	FS	CT	None	78	4
11 [†]	15, 1	F	AR	CC	PPI	62	0
12 [†]	3, 5	M	AR, IA, FA	CT	None	62	0
13 [†]	5, 11	M	None	CT	Sulfasalazine, 6-mercaptopurine, Folic acid	90	0
14 [†]	7, 2	M	AR, FA	CC	PPI	53	5
Mean	5, 9					75	2
Non-responders							
1	7, 4	M	FA, AR, PA	ND	PPI, Fluticasone/Salmeterol, Fexofenadine, Mometasone	60	80
2	2, 3	M	None	CT	Ranitidine	68	67
3	5, 6	M	FA, AR	ND	PPI	60	50
4	6, 9	M	FA, IA	ND	None	65	62
5	7, 10	M	FA, AR	CT	PPI, Loratadine	100	75
6	2, 8	M	FS	CT	PPI	100	50
7	5, 8	F	FA, IA	TT	PPI	79	28
8 [†]	7, 8	F	FA, AR	CT	None	125	65

Patients	Age (year, month)	Gender	Atopy [†]	Genotype	Medications	Peak Eos/HPF	
						Pre	Post
9 [‡]	4, 4	M	AR, PA	CT	PPI, Montelukast, Fluticasone	112	105
10 [‡]	5, 7	M	FS	CT	PPI	90	250
Mean	5, 6					86	83

FS, food sensitivity (i.e. IgE to food without immediate hypersensitivity on ingestion); FA, food allergy; AR, allergic rhinitis; IA, intermittent asthma; PA, persistent asthma.

Table 2

Patient genotypes

Patient group	Genotype		
	CC	CT	TT
Responder	6	6	1
Non-responder	0	6	1