

Evaluation of Different Asthma Phenotype Responses to Montelukast Versus Fluticasone Treatment

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We aimed to determine the variability of response to inhaled corticosteroids (ICS) and leukotriene receptor antagonist (LTRA) in asthmatic children according to the patient phenotype. The present study comprised 56 children (mean age, 9.54 [SD, 2.3] years) with moderate persistent asthma and 18 healthy controls of matched age and sex. Asthmatics were allocated randomly to receive either montelukast (5 mg at bed time) or fluticasone propionate (100 µg twice daily) for 28 days. Serum concentrations of inflammatory mediators as soluble interleukin-2 receptor (sIL-2R), soluble intracellular adhesion molecules-1 (sICAM-1), soluble vascular cell adhesion molecules-1 (sVICAM-1), total serum IgE, peripheral blood eosinophils, and forced expiratory volume in 1 second (FEV₁) were done before and after treatment to patients, and done once to controls. Significant increase in all inflammatory mediators, with significant decrease in FEV₁, was detected in asthmatics before treatment compared to controls. Asthmatics presented with cough and wheeze showed significant increase of FEV₁ and significant decrease in eosinophilic percentage in both treated groups, while the differential response between the two medications was found to be insignificant. Those presented with cough and shortness of breath showed significant increase of FEV₁ and significant decrease in eosinophilic percentage in fluticasone-treated group only. Other inflammatory mediators showed insignificant changes between studied groups after intervention. Response to montelukast and fluticasone vary considerably according to the clinical phenotypes of asthma. Response to montelukast may be more effective in asthmatics presented with wheeze compared to those with shortness of breath. Whereas ICS provide clinical benefit in both asthmatic phenotypes.

Introduction

CHRONIC AIRWAY INFLAMMATION CHARACTERIZES persistent asthma. According to current American and British guidelines, inhaled corticosteroids (ICS) are recognized as the preferred long-term control therapy in patients with persistent asthma including children of all ages, with leukotriene antagonists as an alternative or add-on therapy.¹ The preference for ICS is based primarily on evidence from trials comparing mean responses between a treatment group and a control group; however, there is increased appreciation of the considerable inter-individual variability in response to ICS and leukotriene receptor antagonists (LTRA).¹⁻⁵ Thus, it is important to provide information that guides the clinician in selecting

the medication most likely to achieve a favorable response for particular patients.

Few studies have addressed the factors that determine the marked variability in response to asthma control therapy. It is unknown, for example, whether patients who do not respond well to one medication might respond to the other medication.⁵ The choice of a long-term asthma medication should be based on clinical efficacy, patient symptoms and characteristics, previous responses to treatment, patient's ability to take the medications correctly, side effects, and drug safety. Different studies compared the long-term (>4 weeks) treatment using montelukast and ICS on the clinical asthma control measures, pulmonary function tests (PFTs), and inflammatory biomarkers.⁶⁻¹⁰

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Moving asthma therapy from current approach based on mean drug responses to individual patient phenotype is a promising field of research. This may help the pediatrician for identifying the patient phenotype that would serve as indicators for selection of the medication most likely to achieve a favorable response.

We hypothesized that asthmatic children are variable in their response to controller medications based on the clinical phenotype of asthma. Our aim was to detect whether clinical asthma phenotype will affect the response to the main controller medication: LTRA (montelukast) and ICS (fluticasone).

Patients and Methods

The study was a prospective case control study comprised of 56 children with moderate persistent asthma aged 6–14 years (mean age, 9.54 [SD, 2.3] years). The study included 18 healthy controls of matched age and sex without apparent evidence of allergic diseases (mean age 10.61 SD [0.442] years). They underwent to measurement of serum concentrations soluble intracellular adhesion molecules (sICAM), soluble vascular cell adhesion molecules (sVCAM), soluble interleukin-2 receptor (sIL-2R), total serum IgE level, forced expiratory volume in 1 second (FEV₁), and peripheral eosinophilic percentage as a reference for normal values.

Inclusion criteria for asthmatic children were: the presence of typical asthma symptoms, improvement in the prebronchodilator FEV₁ of $\geq 12\%$ after administration of salbutamol (200 μg), and positive skin prick test. We defined the clinical severity of asthma according to established guidelines of Global Strategy for Asthma Management and Prevention.¹¹ Exclusion criteria was: asthmatic children on immunotherapy.

Skin prick tests were performed only to asthmatic group with different antigens, including house dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*), cat and dog epithelial cells, and mold and pollen antigens (Omega, Canada), together with negative (saline) and positive (0.5% histamine HCl) controls. A mean weal diameter of ≥ 3 mm at 15 min was defined as a positive response.¹² Children were considered atopic if they had at least one positive skin prick test response.

According to the clinical phenotypes, asthmatic children were divided into 32 children presented with cough and wheezes (defined by the patient or the caregiver as creaking, rattle, whistling, jingling) with a mean age of 9.3 (SD, 2.56) years and 24 children presented with cough and shortness of breath (defined by the patient or the caregiver as chest tightness, labored breathing, difficulty indrawing sufficient breath, heavy breath) with a mean age of 9.6 (SD, 2.27) years (Fig. 1). In either group of patients the presenting clinical phenotype had to be persistent during their follow-up, those who had variable clinical presentation between the phenotypes were excluded.

Based on the medication given, we classify our patients into the following groups:

Group A: Comprised 15 patients of cough and wheezes group treated with montelukast.

Group B: Comprised 12 patients of cough and shortness of breath group treated with montelukast.

Group C: Comprised 17 patients of cough and wheezes group treated with fluticasone.

Group D: Comprised 12 patients of cough and shortness of breath group treated with fluticasone.

Informed consent was obtained from all parents of patients and healthy controls, and approved by Ethical Committee of Mansoura Faculty of Medicine, Egypt. Subject characteristics are shown in Table 1.

Study design

This study was designed to be a randomized, non-blinded, pilot prospective study. Expert attended outpatient clinic of Allergy and Clinical Immunology Unit at Mansoura University Children's Hospital had to define and assign patients to the study according to inclusion and exclusion criteria. Patients were randomized to either line of treatment using sealed envelopes kept in the outpatient clinic. Some patients received montelukast as a 5 mg chewable tablet (Singulair, Merck, White house Station, NJ), once daily at bedtime, for 28 consecutive days, while others received fluticasone as inhaled fluticasone propionate 100 μg twice daily, for 28 consecutive days (Flixotide inhaler, pressurized metered-dose inhaler, Glaxo Wellcome Egypt, under license from Glaxo Wellcome Operations, UK).

Short-acting β_2 agonists (ventolin, Glaxo Wellcome, London, UK) were administered as needed for symptomatic relief. Before enrollment of patients in the study, they have to discontinue the following medications: oral or ICS within 1 month, antihistamines within 3 months.¹³

Before and after treatment, measurement of serum concentrations sICAM, sVCAM, sIL-2R, total serum IgE level, FEV₁, and peripheral eosinophilic percentage were done. Before PFTs, short-acting β_2 agonists were withheld for 12 h. Duration of our treatment was 4 weeks based on: montelukast (peak 2–2.5 h with duration of action of 24 h) and ICS (peak 1–4 weeks and duration of action several days after discontinuation).¹⁴

During treatment, patients were asked to regularly visit the outpatient clinic on weekly bases to evaluate compliance to therapy and degree of asthma control. Evaluation was done using a standardized checklist that has records for frequency of day and night symptoms and severity of exacerbations as well as proper compliance to dose and timing for prescribed drug. Patients were excluded from the study if: they showed deterioration of their clinical status; they accidentally used other controller medication; and they were willing to get back to their regular treatment.

Laboratory tests

Blood samples were obtained from each patient before and after 28 days treatment and taken once from healthy controls. Blood was collected into serum separate tubes in two aliquots. One aliquot was used for complete blood count for eosinophils and expressed in cell/mm³. The other was kept for 30 min to clot, and then centrifuged at 3,000 rpm for 10 min. Aliquots of serum were stored at -70°C before analysis. In each sample, sICAM-1, sVCAM-1, sIL-2R, and total IgE levels were measured by using immunoassay techniques. sICAM-1, sVCAM-1, and sIL-2R were measured by using ELISA KITS from DIACLONE Research, France. Serum IgE levels were measured by using IgE EISA KITS from Aptech Services.¹⁵

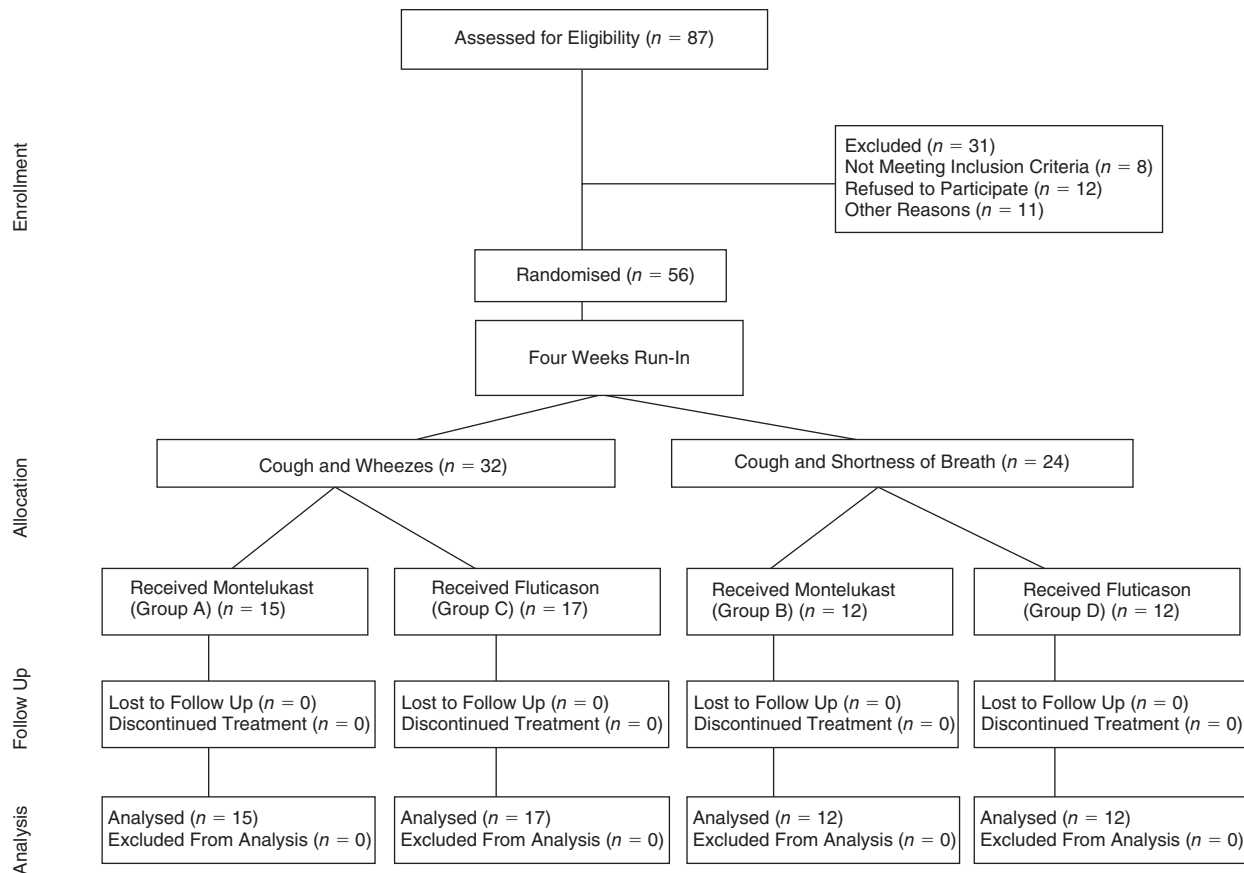


FIG. 1. Flow diagram showing the numbers of children involved in each stage of the study.

Pulmonary function tests (PFTs)

FEV₁ was measured by spirometer (Master Scan body); the highest of three successive measurements was taken. Reference values were computed according to the recommendations of American Thoracic Society (ATS) standards of Acceptability and Reproducibility.¹⁶

Before starting any maneuver, subject data including age, sex, weight, and height must be given to the computer to

allow the computer to calculate the predicted values for this subject. There are three maneuvers done on this apparatus including slow spirometry maneuver, forced spirometry maneuver, and maximal voluntary volume maneuver.

Statistical methods

Statistical analysis was done by using SPSS (Statistical Package for Social Science) software (version 12.0, SPSS Inc,

TABLE 1. DEMOGRAPHIC DATA OF THE STUDIED GROUPS

	Cough and wheezes group (n = 32)	Cough and shortness of breath group (n = 24)	P value
Age in years ^a	9.3 (2.56)	9.6 (2.27)	0.66
Sex			
Male	24 (75%)	13 (53.3%)	
Female	8 (25%)	11 (46.7%)	
Family history			
Positive	19 (59.4%)	14 (58.4%)	
Negative	13 (40.6%)	10 (41.6%)	
Duration of the disease in years ^a	5.22 (2.4)	5.5 (3.4)	0.71
Therapy			
Montelukast	17 (53.1%)	12 (50%)	
Fluticasone	15 (46.9%)	12 (50%)	

^aData are expressed as mean (standard deviation).

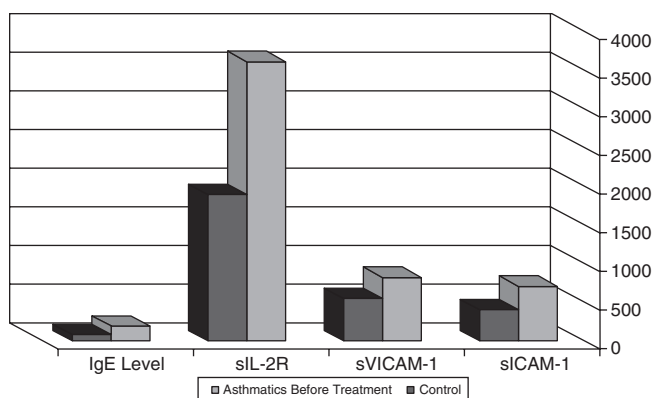


FIG. 2. Showing significant decrease in IgE, sIL-2R, sICAM-1, and sVICAM-1 levels in asthmatic groups before treatment compared with healthy control.

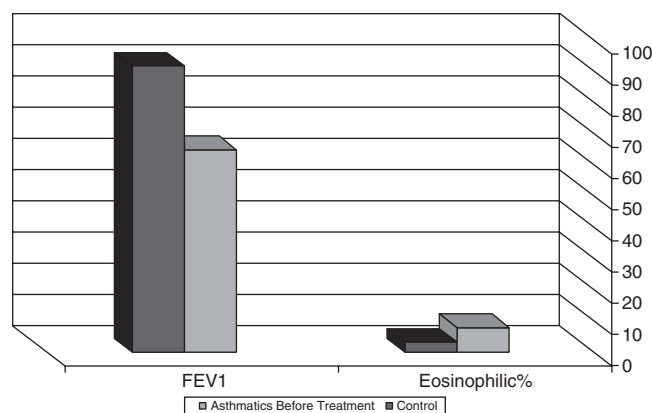


FIG. 3. Showing significant decrease in FEV₁ and significant increase in eosinophilic percentage in asthmatic children compared with healthy controls.

Chicago, IL). The results were analyzed by using: paired *t*-test and Wilcoxon rank serum test to assess differences in levels of sICAM-1, sVICAM-1, sIL-2R, and serum IgE at the beginning and at the end of treatment. Statistical significance was defined as $P < 0.05$.

Results

During the period of recruitment of children to the study, 87 asthmatic patients were presented to the outpatient clinic of Allergy and Clinical Immunology Unit, Mansoura University Children Hospital, 12 children were not included in the study as per their or their parents refusal, eight children were excluded because of exacerbation of symptoms during the 1-month duration of discontinuation of therapy, and 11 patients were excluded because of the poor response or noncompliance to therapy (Fig. 1). Our study involved 56 asthmatic children (mean age 9.54 SD [2.3] years) and 18 healthy controls of matched age and sex. None of the included asthmatic patients had lost follow-up during the study period. Asthmatic children characteristics did not show any significant difference that exists between

asthmatic children presenting with cough and wheeze and those presenting with cough and shortness of breath groups with regard to age and sex (Table 1).

In our study there were statistical significance between asthmatic children and healthy controls regarding serum levels of sICAM-1, sVICAM-1, sIL-2R, total serum IgE, eosinophilic percentage, and FEV₁ (Figs. 2 and 3). Asthmatic children presented with cough and wheeze showed significant improvement of FEV₁ at 4 weeks in both montelukast-treated group (65.9% at baseline to 79.7%, $P < 0.05$) and fluticasone-treated group (65.8% at baseline to 82.3% at 4 weeks, $P < 0.05$); comparing the differential response between the two medications was found to be insignificant. In the same group of children, eosinophilic percentage showed significant decrease from baseline at 4 weeks of therapy in both montelukast-treated group (7.8% to 5.6%, $P < 0.05$) and fluticasone-treated group (8.8%–6.7%, $P < 0.05$). The differential response between both medications was found to be insignificant (Table 2).

Asthmatic children presented with cough and shortness of breath showed significant improvement in FEV₁ only in fluticasone-treated group (66.7%–86.2%, $P < 0.05$), while it

TABLE 2. EFFECT OF MONTELUKAST VERSUS FLUTICASONE ON THE IMMUNOLOGICAL PROFILES AND PULMONARY FUNCTION OF PATIENTS PRESENTED WITH COUGH AND WHEEZES

	Group A			Group C			P
	Before treatment	After treatment	Percentage of change	Before treatment	After treatment	Percentage of change	
sICAM-1	702.2 (45.7)	679.6 (68.2)	−10.6	731.5 (67.2)	622.4 (47.3*)	−7.4	0.22
sVICAM-1	931 (264.3)	853.1 (239.7)	1.3	817.6 (221)	728.4 (245)	−9.39	1.1
sIL-2R	3,976 (523.9)	3,961.4 (498.6)	0.56	3,531.6 (564.2)	3,465.6 (515.7)	−3.58	0.2
IgE level	187.2 (29.7)	182.6 (18.2)	−1.1	180.8 (57.3)	192.4 (40.2)	−3.58	0.8
Eosinophilic percentage	7.8 (1.7)	5.6 (3.5*)	−39.36	8.8 (2.5)	6.7 (2.6*)	−23.67	0.9
FEV ₁	65.9 (3.3)	79.7 (11.3*)	29.22	65.8 (8.1)	82.3 (11.8*)	27.5	0.9

Data are expressed as mean (SD).

* $P < 0.05$ is significant (for each group before and after treatment).

Mann-Whitney *U*-test used to compare both groups.

TABLE 3. EFFECT OF MONTELUKAST VERSUS FLUTICASONE ON THE IMMUNOLOGICAL PROFILES AND PULMONARY FUNCTION OF PATIENTS PRESENTED WITH COUGH AND SHORTNESS OF BREATH

	Group B			Group D			P
	Before treatment	After treatment	Percentage of change	Before treatment	After treatment	Percentage of change	
sICAM-1	701.2 (67.6)	689.6 (64.6)	-14.7	732.3 (86.3)	618.6 (81.1*)	-11.4	0.54
sVICAM-1	943 (327.5)	841.7 (253.3)	11.4	800.8 (211.5)	738 (189.4)	6.4	0.6
sIL-2R	3,673.3 (196.7)	3,470.5 (20.1)	3.8	4,003 (552.4)	3,577.4 (604.7)	1.98	0.53
IgE level	171.8 (36.4)	162.1 (31.1)	-7.1	207.7 (36.5)	145.6 (52.1)	-6.5	0.8
Eosinophilic percentage	6.1 (2.6)	5.6 (3.5)	-1.4	8.7 (1.9)	5.6 (3*)	-9	0.74
FEV ₁	61.7 (9.3)	75 (13.3)	32.2	66.7 (9.2)	86.2 (12*)	32.8	0.88

Data are expressed as mean (SD).

* $P < 0.05$ is significant (for each group before and after treatment).

Mann-Whitney U -test used to compare both groups.

was insignificant in montelukast-treated group (61.7%–75%). The same group of children showed significant decrease of eosinophilic percentage by 9% ($P < 0.05$) in the fluticasone-treated group, while insignificant decrease by 1.4% was noted in the montelukast-treated group (Table 3).

Discussion

Our study aimed to compare the efficacy of LTRA (montelukast) with ICS (fluticasone) on a wide range of airway inflammatory markers and pulmonary function in asthmatic children with different clinical phenotypes. This may serve as indicator for selection of medications most likely to achieve a favorable response. In previous studies, only a single serum marker or clinical parameter was assessed as: serum IgE,¹³ FEV₁,¹³ FEV₁ and symptom score.¹⁷

Our study has shown a wide variability in the pulmonary responses and inflammatory biomarkers to the two asthma controller medications (fluticasone and montelukast) in a population of asthmatic children with different clinical phenotypes. We observed that montelukast significantly improve FEV₁ and significantly decrease eosinophilic percentage in asthmatic children presented with cough and wheeze, whereas it fails to achieve the same response in patients with cough and shortness of breath. On the other aspect, asthmatic children presented with cough and shortness of breath treated with fluticasone showed significant decrease in sICAM-1 and peripheral eosinophilic percentage with significant increase in FEV₁, whereas montelukast failed to achieve these results in asthmatic children with the same clinical phenotypes. This discordance in outcome measures between patients with wheeze and those with shortness of breath may be due to differences among patients in leukotriene biology, genotype, or clinical characteristics.

Why are antileukotriene drugs effective in some patients and ineffective in others? One explanation for the efficacy of these drugs is that Type 1 cysteinyl leukotriene receptor (CysLT1) signaling contributes to many aspects of asthma pathogenesis besides constriction of bronchial smooth muscle. For example, CysLT1 influences systemic immune responses and has bidirectional interactions with Th2 cytokine interleukin-13. IL-13 that participates in the development of asthma can up-regulate both the leukocyte

biosynthesis of leukotriene D4 (LTD4) and the expression of CysLT1. Moreover, LTD4 can up-regulate production of IL-13 as well as the expression of its receptors.¹⁸ Another explanation was given by Drazen et al. who stated that polymorphisms in the promoter of the 5-lipoxygenase gene (ALOX5) were associated with diminished improvement in airflow.¹⁹ However, since these genetic variants occur in <10% of patients with asthma, they can explain only a fraction of the unresponsiveness to leukotriene modifiers. Since levels of leukotrienes and their receptors are greatly influenced by substances such as cytokines, analysis of responsiveness to antileukotriene therapy must take into account the genes for molecules that reside outside the leukotriene pathway.

Our study demonstrated that in asthmatic patients presented with wheeze, both montelukast and fluticasone decrease the peripheral blood eosinophilic percentage and improve FEV₁ significantly from baseline over the 4-week period with insignificant percentage of differential response between the two medications. This demonstrates that orally administered montelukast was not inferior to inhaled fluticasone in these specific asthma phenotypes that presented with wheeze.

Collectively, these data hypothesize that response to montelukast and fluticasone vary considerably according to the clinical phenotypes of asthmatic patient. Response to montelukast may be more effective in asthmatic children presented with wheeze in comparison to those presented with shortness of breath, whereas ICS provide favorable clinical benefit in both asthmatic phenotypes. A big randomized control trial needs to be conducted to explore the impact of asthma clinical phenotypes on the response to controller medications.

References

1. National Asthma Education and Prevention Program National Asthma Education and Prevention Program. Expert Panel Report: Guidelines for the Diagnosis and Management of Asthma Update on Selected Topics—2002. *J Allergy Clin Immunol* 2002; 110:S141–S219.
2. Israel E, Chervinsky PS, Friedman B, Van Bavel J, Skalky CS, Ghannam AE, Bird SR, Edelman JM. Effects of montelukast and beclomethasone on airway function and asthma control. *J Allergy Clin Immunol* 2002; 110:847–854.

3. Malmstrom K, Rodriguez-Gomez G, Guerra J, Villaran C, Piñeiro A, Wei LX, Seidenberg BC, Reiss TF. Oral montelukast, inhaled beclomethasone, and placebo for chronic asthma. A randomized, controlled trial. Montelukast/Beclomethasone Study Group. *Ann Intern Med* 1999; 130:487–495.
4. Simons FE, Villa JR, Lee BW, Teper AM, Lyttle B, Aristizabal G, Laessig W, Schuster A, Perez-Frias J, Sekerel BE, Menten J, Leff JA. Montelukast added to budesonide in children with persistent asthma: a randomized, double-blind, crossover study. *J Pediatr* 2001; 138:694–698.
5. Szeffler SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, Craig TJ, Dolovich M, Drazen JM, Fagan JK, Fahy JV, Fish JE, Ford JG, Israel E, Kiley J, Kraft M, Lazarus SC, Lemanske RF Jr, Mauger E, Peters SP, Sorkness CA; Asthma Clinical Research Network of the National Heart Lung, and Blood Institute. Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002; 109:410–418.
6. Garcia Garcia ML, Wahn U, Gilles L, Swern A, Tozzi CA, Polos P. Montelukast, compared with fluticasone, for control of asthma among 6- to 14-year-old patients with mild asthma: the MOSAIC study. *Pediatrics* 2005; 116:360–369.
7. Ostrom NK, Decotiis BA, Lincourt WR, Edwards LD, Hanson KM, Carranza Rosenzweig JR, Crim C. Comparative efficacy and safety of low-dose Fluticasone propionate and montelukast in children with persistent asthma. *J Pediatr* 2005; 147: 213–220.
8. Sorkness CA, Lemanske RF Jr, Mauger DT, Boehmer SJ, Chinchilli VM, Martinez FD, Strunk RC, Szeffler SJ, Zeiger RS, Bacharier LB, Bloomberg GR, Covar RA, Guilbert TW, Heldt G, Larsen G, Mellon MH, Morgan WJ, Moss MH, Spahn JD, Taussig LM; Childhood Asthma Research and Education Network of the National Heart, Lung, and Blood Institute. Long term comparison of 3 controller regimens for mild moderate persistent childhood asthma: the Pediatric Asthma Controller Trial. *J Allergy Clin Immunol* 2007; 119:64–72.
9. Szeffler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC, Zeiger RS, Larsen G, Spahn JD, Bacharier LB, Bloomberg GR, Guilbert TW, Heldt G, Morgan WJ, Moss MH, Sorkness CA, Taussig LM. Characterization of within subject responses to fluticasone and montelukast in childhood asthma. *J Allergy Clin Immunol* 2005; 115:233–242.
10. Zeiger RS, Szeffler SJ, Phillips BR, Schatz M, Martinez FD, Chinchilli VM, Lemanske RF Jr, Strunk RC, Larsen G, Spahn JD, Bacharier LB, Bloomberg GR, Guilbert TW, Heldt G, Morgan WJ, Moss MH, Sorkness CA, Taussig LM; Childhood Asthma Research and Education Network of the National Heart, Lung, and Blood Institute. Response profiles to fluticasone and montelukast in mild-to-moderate persistent childhood asthma. *J Allergy Clin Immunol* 2006; 117:45–52.
11. Global Strategy for Asthma Management and Prevention, NHLBI/WHO workshop report. Publication No. 02-3659, Bethesda, MD: National Institutes of Health, National Heart, Lung, and Blood Institute, 2002.
12. Brown WG, Halonen MJ, Kaltenborn WT, Barbee RA. The relationship of respiratory allergy, skin test reactivity, and serum IgE in a community population sample. *J Allergy Clin Immunol* 1979; 63:328–335.
13. Stelmach I, Bobrowska-Korzeniowska M, Majak P, Stelmach W, Kuna P. The effect of montelukast and different doses of budesonide on IgE serum levels and clinical parameters in children with newly diagnosed asthma. *Pulm Pharmacol Ther* 2005; 18:374–380.
14. Deglin JH, Vallerand AH (Eds.). *Davis's Drug Guide for Physicians*. Philadelphia: F.A. Davis Company, 2007 [Electronic Book]. <http://www.skyscape.com/estore/ProductDetail.aspx?ProductId=220>.
15. Ishizaka T. IgE and mechanisms of IgE mediated hypersensitivity. *Ann Allergy* 1982; 48:313–319.
16. Crapo RO, Hankinson JL, Irvin C. Standardization of spirometry. *Am J Respir Crit Care Med* 1995; 152:1107–1136.
17. Karaman O, Sünneli L, Uzuner N, Islekel H, Turgut CS, Köse S, Tezcan D, Coker C, Erbayraktar Z. Evaluation of montelukast in 8–14 year old children with mild persistent asthma and compared with inhaled corticosteroids. *Allergol Immunopathol (Madr)* 2004; 32:21–27.
18. Peters-Golden M, Henderson WR Jr. Leukotrienes. *N Engl J Med* 2007; 357:1841–1854.
19. Drazen JM, Yandava CN, Dubé L, Szczerback N, Hippensteel R, Pillari A, Israel E, Schork N, Silverman ES, Katz DA, Drajesk J. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nat Genet* 1999; 22:168–170.

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