

Exposure to tobacco smoke increases leukotriene E₄-related albuterol usage and response to montelukast

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Background: Cysteinyl leukotrienes (CysLTs) are important mediators of asthma in children. Predictors of susceptibility to CysLT effects have not been developed.

Objectives: To identify susceptibility markers to CysLT effects and montelukast response.

Methods: Twenty-seven schoolchildren were followed for 5 months with measurements of urinary leukotriene E₄ (LTE₄), cotinine, fractional exhaled nitric oxide (FENO), and monitoring of albuterol use. After a baseline run-in, children were randomized to receive daily montelukast or placebo without change in their current controller medications.

Results: At baseline, a significant ($P = .003$) positive association was observed between LTE₄ levels and albuterol use 2 days later. LTE₄-related albuterol usage (ie, change per interquartile increase in LTE₄) declined significantly after montelukast treatment (12% decline; $P = .0005$ for relative difference between intervals) but not placebo (2% increase; $P = .80$).

Declines in LTE₄-related albuterol usage between intervals tended to be greater in girls ($P = .01$ for girls; $P = .21$ for boys; $P = .07$ for interaction) and were greater among children with higher cotinine levels ($P = .01$ for high cotinine group; $P = .17$ for low cotinine group; $P = .04$ for interaction). Children with high LTE₄ levels relative to FENO demonstrated significant ($P = .05$) declines in LTE₄-related albuterol usage between intervals ($P = .89$ for low ratio group; $P = .25$ for interaction).

Conclusion: Increased individual CysLT levels are associated with subsequent albuterol usage. CysLT-related albuterol usage and montelukast responsiveness are increased in children exposed to tobacco smoke and tend to be greater in girls than boys. Measurement of LTE₄ to FENO ratios may help predict susceptibility to montelukast. (J Allergy Clin Immunol 2008;121:1009-1016.)

Key words: Asthma, children, cysteinyl leukotrienes, LTE₄, FENO, montelukast

The significance of cysteinyl leukotrienes (CysLTs) as key mediators and modulators in the pathogenesis of allergic airway

Abbreviations used

CysLT: Cysteinyl leukotriene
ETS: Environmental tobacco smoke
FENO: Fractional exhaled nitric oxide
GEE: Generalized estimating equation
ICS: Inhaled corticosteroid
IQR: Interquartile range
LABA: Long-acting β -agonist
LTE₄: Leukotriene E₄
LTRA: Leukotriene receptor antagonist

disease has been reported in both experimental and clinical settings. Inhaled leukotriene D₄ is a bronchoconstrictor 10,000 times more potent than histamine or methacholine.^{1,2} In addition to their potent bronchoconstrictive properties, CysLTs induce other pathophysiological responses characteristic of the inflammatory response in asthma.^{3,4} Clinical studies have demonstrated that leukotriene receptor antagonists (LTRAs) are able to reduce rescue treatment requirements, improve pulmonary function, and reduce symptoms in adults and children with asthma.^{5,6}

This relationship between CysLTs and asthma severity appears to not be homogenous across populations. For example, a number of studies have reported that female schoolchildren respond more favorably to montelukast than boys^{7,8} and that smoking adults show a greater response than nonsmokers.⁹ Why antileukotriene medications are effective in some patients and ineffective in others remains unclear. Some studies suggest that susceptibility to LTRAs is related to differences in CysLT levels between individuals,^{7,10} whereas other studies have not observed this relationship.¹¹ As such, it is unclear whether susceptibility is primarily a function of increased CysLT production, differences in pharmacokinetic metabolism of the medication, increased receptor sensitivity, or perhaps a more complex interaction with other mediator pathways. Identifying biological and phenotypic characteristics related to CysLT effects and efficacy of montelukast would allow for more defined clinical evaluations focused on subpopulations most likely to benefit from such treatments.

In a previous study,¹² the relationship between the stable end-product of CysLT metabolism, leukotriene E₄ (LTE₄), and asthma control was examined. Among a cohort of children predominantly receiving inhaled corticosteroid (ICS), significant relationships were observed between increases in urinary LTE₄ and loss of asthma control. The current study uses a similar but experimental model with a placebo-controlled intervention to identify biological and subject-level predictors of increased individual susceptibility to the effects of CysLTs and montelukast treatment among schoolchildren with asthma.

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TABLE I. Demographics

Group	No.	Age (y), range	Male, no. (%)	Symptom nights/wk, no. (%) [*]	As-needed albuterol days/wk, no. (%) [*]	ICS usage (includes ICS and LABA), no. (%)	ICS and LABA only, no. (%)
Montelukast	14	11 (7-15)	8 (57)	<2:7 (50)	0:4 (28)	12 (86)	7 (50)
				2-3:4 (29)	1-2:8 (57)		
				>3:3 (21)	>2:2 (14)		
Placebo	13	11 (7-13)	11 (85)	<2:4 (31)	0:3 (23)	10 (77)	3 (23)
				2-3:7 (54)	1-2:8 (61)		
				>3:2 (15)	>2:2 (15)		

^{*}Previous year's average.

METHODS

Subjects

Twenty-eight children, age 6 to 15 years, who attended the Kunsberg School at the National Jewish Medical and Research Center and had physician-diagnosed asthma were followed over a 5-month period (November 15, 2005, to April 15, 2006). Determination of adequate sample size for this study was based on numbers from the previous related study.¹² As such, it was determined that significant interactions with LTE₄-related asthma control would be observed in a study with a similar number of subjects and observations.

Children were excluded from the study if they were already receiving montelukast. The Kunsberg School is a public elementary school designed to address the educational needs of children with significant asthma that interferes with regular school attendance and progress. Many of the children are classified as urban poor, with approximately 2/3 of the students receiving Medicaid assistance. Ethical and scientific approval for this study was obtained from the National Jewish Institutional Review Board before initiation of recruitment.

Protocol

After an initial 2½-month period (baseline interval), children were randomized to receive daily montelukast (montelukast group) or placebo (placebo group) for the final 2½ months of the study (treatment interval). Study medication and placebo were administered in a single-blind fashion by school nurses or study personnel on school days and by parents (who were blinded) on nonschool days. Children were otherwise followed by their regular physicians, who were free to adjust medications as needed (these medication changes were recorded and controlled for in the statistical analysis).

Medication monitoring

Each child was given a Doser (Meditrack, Hudson, Miss) for use at school throughout the 5-month period. The Doser is an electronic counter that records the total number of (albuterol) activations in each 24-hour period. At study initiation, parents completed a questionnaire describing baseline demographic and asthma severity characteristics. School nurses recorded all other medication changes during the study.

Urine collection

Sixteen urine samples were collected from each child on 8 consecutive school days during the baseline interval and 8 consecutive school days during the treatment interval. Urine was collected at approximately the same time each day (10:00 AM to 12:00 PM). Collection was staggered in groups of 10 children so that urine monitoring occurred daily throughout the 5 months of the study.

LTE₄ and cotinine measurements

Urineary LTE₄ and cotinine were measured as previously described.^{13,14} Urine LTE₄ levels were reported in picograms and standardized per milligram of creatinine. Urine cotinine levels were reported in nanograms per milliliter and standardized per milligram of creatinine.

Fractional exhaled nitric oxide measurement

Each child performed up to 16 fractional exhaled nitric oxide (FENO) measurements on a NIOX device (Aerocrine, Stockholm, Sweden) at

approximately the same time as urine collection. Standard flow-rates (50 L/s) and length of collection (6 seconds) were used.¹⁵

Statistical analysis

Baseline model. A Poisson regression model using generalized estimating equations (GEEs)¹⁶ was used to determine the strength of association between albuterol medication use (modeled as the outcome) and LTE₄ levels at various lag times (in days) between the variables. An indicator for Friday (1 if response was on Friday, 0 otherwise) was included because children had regular exercise activities from Monday through Thursday, but not on Friday. Consequently, subjects used significantly less albuterol on Fridays. The same day and a lag as long as 4 days of LTE₄ was tested for, in separate models.

Experiment model. Daily albuterol use was modeled as a function of LTE₄, group (montelukast and placebo), interval (baseline and treatment), and all interactions of these variables, with Poisson regression and GEE. With the baseline model as a guide, 3-day moving averages of LTE₄ (days 0-2 and 1-3 before current day) were used. Other covariates in the model included daily indicators for Friday, prednisone use, special exercise days during the treatment interval, and interval level indicators of ICS and LABA use.

Effect modifier models. These models were fit for the subjects randomized to montelukast only. The model had LTE₄, interval, the effect modifier of interest, and all interactions of these variables. Otherwise, the model was similar to the experiment model described. The effect modifiers tested included sex, ICS and LABA use, mean LTE₄, mean FENO, height, mean cotinine, and mean LTE₄ to FENO ratio.

The interquartile range (IQR) for LTE₄ was used to standardize slope estimates; this statistic was determined by first calculating the IQR for each subject, and then taking the median across subjects. For all models described, a first-order autoregressive working covariance structure was used to model repeated measures within subjects over time. SAS software, version 9.1 (PROC GENMOD; SAS Institute, Cary, NC) was used to fit the data to the models described. Because of the tendency for SEs of β estimates in GEE models to be underestimated for small sample sizes, SEs for all models were adjusted using the Kauermann and Carroll correction factor.^{17,18}

RESULTS

Demographics

All children completed the 5-month protocol. One child, randomized to placebo, was started on montelukast by the child's physician and therefore was not included in the analysis. Table I summarizes demographic and asthma severity information in the remaining children based on a prestudy questionnaire. Montelukast and placebo groups were comparable with respect to these variables including sex ($P = .21$) and symptom severity ($P = .53$).

Biomarker levels

Leukotriene E₄ measurements were performed on 387 collected urine samples (186 and 201 in the baseline and treatment intervals, respectively), and there were 386 FENO measurements (Table II). Across groups and intervals, the median subject IQR

TABLE II. Median and IQR statistics for LTE₄, FENO, cotinine, and LTE₄/FENO ratios by treatment group and interval

Variable	Placebo, baseline	Montelukast, baseline	P value*	Placebo, treatment	Montelukast, treatment	P value*
LTE ₄ †	69.6 (34.0)	75.4 (33.5)	.98	87.1 (24.0)	78.9 (29.5)	.73
FENO†	20.7 (3.85)	31.6 (12.9)	.07	19.4 (4.14)	34.9 (13.3)	.14
Cotinine†	1.39 (0.7)	3.57 (1.55)	.11	1.63 (1.00)	3.08 (2.2)	.37
LTE ₄ /FENO‡	4.87 (2.06)	2.63 (2.17)	.05			

*Between-group difference for the interval, using a Wilcoxon rank-sum test.

†Median subject average (median subject IQR). Statistics were determined by calculating averages and IQRs within subjects first, and then taking the median across subjects.

‡Median LTE₄/FENO ratio (IQR LTE₄/FENO ratio). The ratio variable was calculated for each subject as the mean LTE₄, divided by the mean FENO, using all values during the interval. Median and IQR statistics were then calculated across subjects.

for LTE₄ was 31 pg/mg creatinine, which was used as the standard increase for albuterol change estimates.

Relationship between albuterol use and LTE₄ increases

During the baseline interval, mean (SD) albuterol puffs per day was 1.8 (1.1) for the placebo group and 2.2 (0.8) for the montelukast group. Urine LTE₄ levels measured in all subjects during the baseline interval were strongly associated with increased albuterol use (21.9% increase in albuterol usage per LTE₄ IQR increase; $P = .003$) 2 days after urine collection (day 2; Table III).

Relative changes in albuterol use after montelukast treatment

Next, the efficacy of montelukast treatment on LTE₄-related albuterol usage (ie, increase in albuterol usage per IQR change in LTE₄) was examined. Based on results from the previous analysis, the primary lag structure used in these models was a 3-day moving average, from days 1 to 3, which was centered on the day with the largest observed effect (day 2). Also analyzed was the 3-day moving average from days 0 through 2. The moving average often reduces variability in estimates compared with considering individual days.

For the days 1 to 3 moving average, albuterol usage increased 11.3% per IQR increase in LTE₄ ($P = .04$) during the baseline interval for the montelukast group (Table IV). During the treatment interval, this relationship was not apparent (-3.2% ; $P = .44$), and the relative change in associations between intervals for this group was significant ($P = .0005$). For children in the placebo group, albuterol usage per IQR increase in LTE₄ was 4.6% and 7.0% in the baseline and treatment intervals, respectively ($P = .80$ for relative difference).

Using the days 0 to 2 moving average, albuterol usage increased 20.5% ($P < .01$) and 8.8% ($P = .24$) per IQR increase in LTE₄ for the placebo and montelukast groups, respectively, during the baseline period. Similar statistics were not significant during the treatment interval for either group (5.9%, $P = .56$ for placebo; -3.0% , $P = .50$ for montelukast). Despite the large baseline value for the placebo group, the relative difference in statistics between intervals was not significant for the placebo group ($P = .19$), whereas the relative difference was significant for the montelukast group ($P = .05$).

Predictors of susceptibility

Using the days 1 to 3 moving average effect model, which had provided the most robust estimates in the experiment model,

TABLE III. Albuterol usage change per LTE₄ IQR increase during the baseline interval (all subjects)

Day no.	No. of subject-day observations	Change in albuterol usage per LTE ₄ IQR (95% CI)	P value
0	186	7.5% (0.4, 15.1)	.04
1	148	9.2% (-1.7 , 21.2)	.10
2	108	21.9% (7.0, 39.0)	.003
3	82	3.5% (-5.9 , 13.8)	.48
4	68	5.8% (-3.3 , 15.8)	.22

predictors of susceptibility to CysLT-related albuterol usage and to montelukast treatment were then assessed. Seven potential effect modifiers were selected on the basis of susceptibility predictors reported in previous studies. These were use of combination controller medication (ie, ICS with LABA),⁸ height or age,^{7,19} sex,^{7,8} tobacco exposure as measured by mean urine cotinine levels,⁹ mean LTE₄ level,^{7,10} mean FENO level,^{7,20} and mean LTE₄ to FENO level ratio level. Values used were from the baseline interval only to ensure that these levels were unaffected by montelukast treatment. Estimates of all these variables are summarized in Table V.

Demographic markers. Among the variables tested, sex was a near-significant modifier whereas cotinine levels were significant modifiers of the montelukast and LTE₄-related albuterol relationship. During the baseline interval, girls demonstrated a 20.7% increase in albuterol usage per LTE₄ IQR ($P = .004$), whereas boys' relative albuterol usage increased by 8.0% ($P = .32$). Reflecting these differences in CysLT-related albuterol usage, declines in LTE₄-related albuterol usage with montelukast treatment tended to be greater in girls than boys ($P = .01$ for girls; $P = .21$ for boys; $P = .07$ for between-group interaction comparing declines; Fig 1, A). Children in the 3rd quartile for urine cotinine demonstrated an 11.8% increase in albuterol usage per LTE₄ IQR at baseline ($P = .07$), whereas LTE₄-related albuterol usage was increased by 4.6% among children in the 1st cotinine quartile ($P = .41$). Declines in LTE₄-related albuterol usage after montelukast treatment were significant ($P = .01$) among children with higher cotinine but not the lower cotinine quartile ($P = .18$ for low cotinine group), and the interaction between groups was significant ($P = .04$ for interaction between groups; Fig 1, B).

Biomarkers. During the baseline interval, LTE₄ levels were significantly but weakly correlated with same-day FENO levels ($r = 0.38$ for placebo group, $P = .0003$; $r = 0.27$, $P = .015$ for the montelukast group). Mean LTE₄ levels did not predict susceptibility, nor were any trends observed at baseline or after treatment. LTE₄-related albuterol usage at baseline was near significant for children in the 1st FENO quartile (11.5% increase per LTE₄ IQR, $P = .07$ for 1st quartile; 5.9% decrease, $P = .74$ for 3rd

TABLE IV. Change in albuterol usage per IQR increase in LTE₄ (95% CI; entries are expressed as %)

Group	Lag 1-3				Lag 0-2			
	Baseline interval	Treatment interval	Relative difference*	P value†	Baseline interval	Treatment interval	Relative difference*	P value†
Montelukast	11.3 (0.4, 23.4)	-3.2 (-10.8, 5.1)	-13.0 (-19.6, -5.9)	.0005	8.8 (-5.4, 25.1)	-3.0 (-11.2, 5.9)	-10.8 (-20.3, -0.1)	.05
Placebo	4.6 (-4.8, 15.0)	7.0 (-4.9, 20.3)	2.2 (-13.4, 20.7)	.80	20.5 (3.0, 41.1)	5.9 (-12.8, 28.6)	-12.1 (-27.6, 6.7)	.19

*Determined as the fold change in albuterol usage per IQR increase in the treatment interval, divided by the fold change in albuterol usage per IQR increase in the baseline interval, and expressed here as percentage change by subtracting 1.

†P value for relative difference estimate.

TABLE V. Predictors of susceptibility to montelukast

Predictor	Interval or comparison	Group 1 or 1st quartile estimate of change in albuterol usage per IQR of LTE ₄ , expressed as %	Group 2 or 3rd quartile estimate of change in albuterol usage per IQR of LTE ₄ , expressed as %	Effect modifier P value‡
No medications or ICS (group 1 meds) vs ICS and LABA (group 2 medications)	Baseline	14.8 (P = .11)	6.0 (P = .56)	.27
	Treatment	-10.5 (P = .35)	-1.0 (P = .75)	
	Relative difference†	-22.0 (P = .10)	-6.6 (P = .36)	
Height	Baseline	16.7 (P = .12)	19.3 (P = .09*)	.99
	Treatment	-2.4 (P = .57)	-0.3 (P = .96)	
	Relative difference†	-16.4 (P = .04**)	-16.4 (P = .04**)	
LTE ₄	Baseline	13.7 (P = .35)	6.8 (P = .45)	.83
	Treatment	-1.5 (P = .92)	-4.8 (P = .47)	
	Relative difference†	-13.4 (P = .36)	-10.9 (P = .25)	
FENO	Baseline	11.5 (P = .07*)	-5.9 (P = .74)	.68
	Treatment	-0.1 (P = .99)	-6.4 (P = .22)	
	Relative difference†	-10.4 (P = .17)	-6.6 (P = .98)	
LTE ₄ :FENO ratio	Baseline	-2.6 (P = .79)	19.7 (P = .03**)	.25
	Treatment	-0.8 (P = .87)	-1.3 (P = .80)	
	Relative difference†	1.8 (P = .89)	-17.6 (P = .05*)	
Male (group 1 sex) vs female (group 2 sex)	Baseline	8.0 (P = .32)	20.7 (P = .004**)	.07*
	Treatment	-0.5 (P = .92)	-11.5 (P = .25)	
	Relative difference†	-7.9 (P = .21)	-26.7 (P = .01**)	
Cotinine	Baseline	4.6 (P = .41)	11.8 (P = .07*)	.04**
	Treatment	-4.0 (P = .25)	-3.6 (P = .29)	
	Relative difference†	-8.2 (P = .18)	-6.6 (P = .01**)	

*P < .1.

**P < .05.

†Determined as the fold change in albuterol usage per IQR increase in the treatment interval, divided by the fold change in albuterol usage per IQR increase in the baseline interval, and expressed here as percentage change by subtracting 1.

‡LTE₄-by-interval-by-predictor interaction.

quartile), but relative differences between intervals were not significant in either group ($P = .17$ for 1st quartile; $P = .98$ for 3rd quartile; $P = .68$ for between-group interaction).

Children in the 3rd quartile for LTE₄ to FENO ratio demonstrated a 19.7% increase in albuterol usage per LTE₄ IQR at baseline ($P = .03$), whereas LTE₄-related albuterol usage decreased by 2.6% per LTE₄ IQR among children in the 1st ratio quartile ($P = .79$) during this interval. Declines in LTE₄-related albuterol usage after montelukast treatment were significant among children in the 3rd LTE₄ to FENO quartile ($P = .05$) but not the 1st quartile ($P = .89$). However, between-group interactions for these declines were not significantly different ($P = .25$; Fig 1, C).

DISCUSSION

Because asthma involves multiple mediators, the efficacy of LTRAs, which target only the CysLT pathway, will depend on

certain asthma phenotypes in which CysLTs play a predominant role in disease pathogenesis. In this randomized, placebo-controlled study, increased albuterol usage among schoolchildren was associated with changes in CysLT levels (as defined by urinary LTE₄ excretion) and attenuated after randomization to treatment with montelukast. Of interest, the strength of this CysLT-albuterol interaction at baseline and subsequent response to montelukast was not uniform over all groups. Responses tended to be greater in girls than boys, and children exposed to higher levels of environmental tobacco smoke (ETS) demonstrated greater susceptibility to montelukast than those with lower ETS exposures.

These findings suggest that changes in CysLT levels in certain individuals are related to asthma control. In this context, children received 22% more albuterol per IQR increase in LTE₄. Because the approximate mean albuterol use in these children was 2 puffs per day, on average, children received 13 extra puffs per month when LTE₄ levels were high compared with when they were

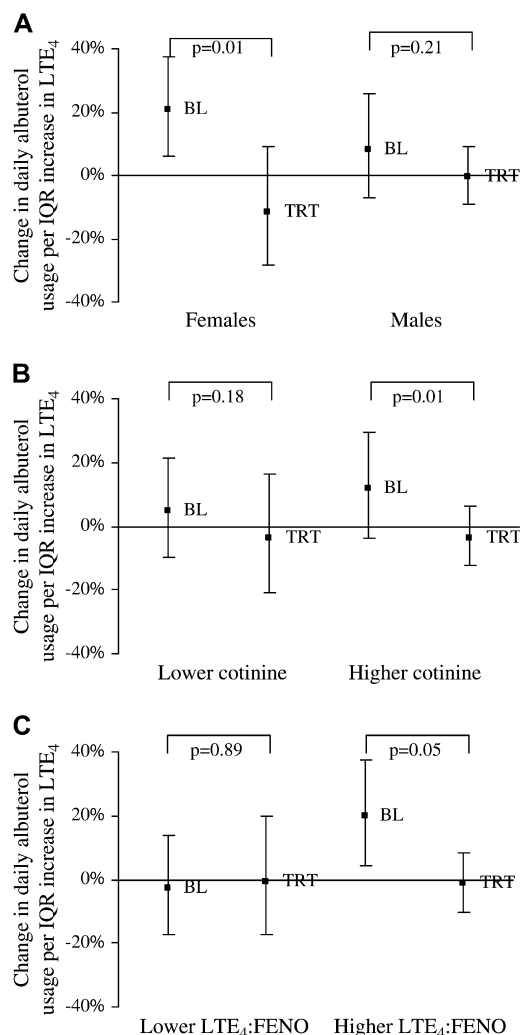


FIG 1. Effect modifiers of the montelukast effect on LTE_4 -associated albuterol usage. Shown are mean estimates and 95% CIs for change in albuterol use per IQR increase in LTE_4 , modified by sex (**A**), mean cotinine (**B**), and mean LTE_4 to mean FENO ratio (**C**). Estimates are given for baseline (BL) and treatment (TRT) intervals for subjects in the montelukast group. The relationship is based on albuterol use for a given day, and the average of LTE_4 measurements over the previous 1 to 3 days. **B** and **C**, Lower and higher values of effect modifiers were based on estimates at first and third quartiles, respectively.

low. In the current study, children were followed in a relatively well controlled setting over consecutive days. As such, there was limited variability in LTE_4 and albuterol usage. Assuming a linear relationship, this percent change relationship could be extrapolated to different settings to estimate, for example, the magnitude of LTE_4 increase in predicting an asthma exacerbation.

The temporal relationship between urine LTE_4 and increased albuterol use suggests an important delayed or accumulated temporal response similar to what we reported in a previous observational study, in which a 1-day delayed effect was observed.¹² Similar but more limited results have been reported in other observational time-series studies.^{21,22} In this respect, this prospective placebo-controlled study confirmed in a randomized model the importance of individual CysLT variations with subsequent albuterol usage and the effectiveness of montelukast treatment in blunting this response.

By using a repeated-measures model, this study was able to illustrate how between-subject variability in CysLT effects might be related to the heterogeneous response observed with montelukast treatment. As such, increases in urinary LTE_4 during the baseline interval were related to increased albuterol usage only among certain subgroups of children. Consequently, after montelukast treatment was initiated, there were significant changes in LTE_4 -related albuterol usage in some and little change in other children. In this context, girls demonstrated significantly increased LTE_4 -related albuterol usage during the baseline interval. During the subsequent treatment interval, this association was significantly attenuated in girls only, and near-significant modification of this attenuation effect was observed between female and male groups. This sex-related susceptibility trend is supported by 2 previous pediatric studies. Both Szefer et al⁷ and Johnston et al⁸ reported that school-aged girls were more responsive to montelukast treatment. Such interactions may, in fact, be age-dependant if preschool boys and school-aged girls are more responsive to montelukast, as reported by Johnston et al.⁸ These authors speculated that hormonal influences on the consequences of CysLT receptor ligation might alter susceptibility.

In a recent publication, Lazarus et al⁹ showed that adult smokers with asthma were more responsive to montelukast, whereas nonsmokers responded preferentially to treatment with ICS. In the current study, these potential susceptibility findings were extended for the first time to children exposed to second-hand smoke. Together, the 2 studies indicate that tobacco smoke exposure may trigger asthma worsening through a predominant CysLT-mediated pathway. In this context, studies have reported that tobacco smoke exposure precipitates a biological response in the airway distinct from the conventional allergen-triggered steroid-responsive eosinophilic response.^{23,24} These clinical and experimental studies suggest the presence of distinct biological pathways or end-organ sensitivities in asthma that may differ according to the exposure trigger.

The suggestion of a distinct biological phenotype related to montelukast responsiveness is supported by this study's investigation of the role of urine LTE_4 and FENO as markers of susceptibility. In previous studies, mean differences in LTE_4 levels alone did not consistently predict the presence of asthma²⁵ or associate with asthma severity.²⁶ Similarly, in this study, susceptibility was not related to differences between children in mean LTE_4 levels unless mean FENO levels were concurrently low. Other studies in schoolchildren with mild to moderate asthma have reported that FENO levels are well correlated with classical inflammatory markers related to the allergic response such as peripheral blood eosinophil IgE and serum eosinophil cationic protein and weakly correlated with levels of urinary LTE_4 , indicating that CysLTs may be elevated independent of conventional steroid-responsive eosinophilic inflammation measured by FENO.²⁷ In the current study, children with low FENO relative to urine LTE_4 levels demonstrated significant associations between LTE_4 and albuterol usage during the baseline interval and significant attenuation of these associations during montelukast treatment in a similar fashion as observed in sex-stratified and cotinine-stratified groupings. However, between-group interactions were weaker in this analysis, possibly because of the increased variability in ratio measurements instead of a single outcome. If confirmed in a larger context, the magnitude of the differences between groups indicates a large effect modification on CysLT responsiveness related to this biological pattern. Studies in school-age children by

Szeffler et al⁷ and Zeiger et al²⁰ provide additional support for this interaction pattern, because increased montelukast responsiveness relative to ICS was reported among children with high LTE₄ levels, whereas relatively poor responses to montelukast were observed in children with high IgE, eosinophil cationic protein, and FENO levels.

What mechanism could explain this interaction? Because both nonspecific airway hyperreactivity²⁸ and CysLT receptor density²⁹ tend to increase with elevated levels of FENO-related cytokines, decreased CysLT responsiveness in the setting of higher FENO levels should not be related to increased end-organ sensitivity to CysLTs. Instead, this biological phenotype may indicate the limited benefit of montelukast when CysLTs as well as conventional eosinophil-related mediators are increased in the airway (measurable by higher mean LTE₄ and FENO levels) because of the overlapping effects of multiple mediators. In contrast, when CysLTs are secreted into a more pristine inflammatory milieu with relatively low levels of FENO-related mediators (measurable by higher mean LTE₄ and lower FENO levels), attenuating the effects of CysLTs with montelukast may lead to clinically significant improvement. We speculate that this is the case in children with asthma who are repeatedly exposed to ETS as their primary asthma trigger. In this context, studies have reported both that urinary LTE₄ excretion increases acutely after tobacco smoking³⁰ and that levels of FENO are lower in schoolchildren with chronic ETS exposure.^{31,32} Future studies are planned to assess directly the association between ETS exposure and levels of LTE₄, FENO, and albuterol usage with acute and chronic exposure models.

The observations in this study have a number of clinical applications. Although ICSs are generally considered more potent than LTRAs and are therefore recommended as first-line therapy,³³ treatment with ICS alone may not adequately control disease in 30% to 40% of patients.³⁴ This could reflect the inability of ICS therapy to reduce leukotriene production in certain patient subsets in which this pathway predominates.^{35,36} Although the weight of evidence suggests that LABAs are superior to LTRAs in combination therapy,³⁷ some studies have reported that the addition of montelukast to ICS therapy is similar to LABAs in reducing symptoms and exacerbations of asthma.^{38,39} In certain patients, the choice of montelukast may be warranted, particularly in school-age girls and children exposed to tobacco smoke; determination of LTE₄ to FENO ratios may help predict responsiveness among these and other potentially susceptible groups.

In contradistinction to studies that assess between-subject variability by using averaged outcome measurements, the panel design in this study with multiple repeated measurements is well suited to detect susceptible subgroups or individuals because within-subject exposure-response relationships can be determined with great precision.⁴⁰ This allowed for significant observations despite the small number of subjects. Nevertheless, the size of the sample did not allow for an analysis of the interacting variables to determine with confidence whether these were independent or covarying predictors. This limits to some extent the application of these observations to other clinical settings.

In conclusion, in a randomized, placebo-controlled study, increased albuterol usage was associated with changes in individual LTE₄ levels in a panel of schoolchildren receiving daily ICS therapy. Girls and those with higher cotinine levels were more responsive to LTE₄ changes and more susceptible to

interventions with montelukast. Among subsets of children with asthma on ICS therapy, environmental and subject-level characteristics may lead to a distinctive pathogenic pathway predominantly mediated by CysLTs and susceptible to treatments with LTRAs.

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Clinical implications: Among subsets of children with asthma on ICS therapy, tobacco smoke exposure and sex are related to susceptibility to montelukast treatment. Montelukast susceptibility may be predicted by high LTE₄ to FENO ratios.

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