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REVIEW

Role of biomarkers in understanding and treating children with asthma: towards personalized care

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Abstract: Asthma is one of the most common chronic diseases affecting children. Despite publicized expert panels on asthma management and the availability of high-potency inhaled corticosteroids, asthma continues to pose an enormous burden on quality of life for children. Research into the genetic and molecular origins of asthma are starting to show how distinct disease entities exist within the syndrome of "asthma". Biomarkers can be used to diagnose underlying molecular mechanisms that can predict the natural course of disease or likely response to drug treatment. The progress of personalized medicine in the care of children with asthma is still in its infancy. We are not yet able to apply stratified asthma treatments based on molecular phenotypes, although that time may be fast approaching. This review discusses some of the recent advances in asthma genetics and the use of current biomarkers that can help guide improved treatment. For example, the fraction of expired nitric oxide and serum Immunoglobulin E (IgE) (including allergen-specific IgE), when evaluated in the context of recurrent asthma symptoms, are general predictors of allergic airway inflammation. Biomarker assays for secondhand tobacco smoke exposure and cysteinyl leukotrienes are both promising areas of study that can help personalize management, not just for pharmacologic management, but also education and prevention efforts.

Keywords: asthma, biomarkers, children, management

Introduction

Asthma is one of the most common chronic diseases affecting children. The number of people worldwide affected by asthma may be as high as 300 million.[1] Asthma continues to pose an enormous burden on quality of life and health care costs. Despite widely publicized expert panels on asthma management and the availability of high-potency inhaled corticosteroids, asthma continues to be a leading cause of hospitalization, emergency department visits, and missed school in children.[2] In 2009, one in five of the estimated eight million children in the United States with asthma went to the emergency department for asthma. Asthma has a strong familial aggregation and can present as one of several distinct phenotypes (Table 1). Despite the high degree of heritability, external factors such as air pollution, immune sensitization, secondhand smoke, nutrition, and obesity can affect both disease risk and control of symptoms.

Asthma continues to be a disease that is diagnosed clinically, using history-taking (recurrent wheeze, cough, and dyspnea triggered by viral infections, allergen, exercise or pollutants, and response to glucocorticoids and bronchodilators), physical examination, and pulmonary function testing (reversible airflow obstruction). However, it is becoming clearer that within a population of patients diagnosed with asthma, there

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Table 1 Common phenotypes of childhood asthma

Phenotype	Onset	Natural history	Triggers	Biomarker characteristics	Treatment
Atopic	Infancy	chronic	Virus, allergen	Elevated IgE, FENO	Chronic: ICS
Virus-induced wheezing	Infancy	Resolves by school age	Virus	Normal IgE, FENO	Supportive care, bronchodilators
Exercise-induced	School age – adolescence	chronic	Exercise	IgE, eosinophils,[110] cysLTs[111] may be elevated; FENO typically normal[112]	Acute: Pretreat with bronchodilator, Chronic: montelukast, cardiopulmonary conditioning
Obesity-related	All ages, but more common in adolescence	May resolve with weight loss	Non-specific	Typically normal IgE, normal FENO	Weight loss; ICS may be less effective among obese; consider LTM

Abbreviations: IgE, Immunoglobulin E; FENO, fractional exhaled nitric oxide; ICS, inhaled corticosteroid; cysLT, cysteinyl leukotrienes (LTC4, LTD4, and LTE4); LTM, leukotriene modifier.

exists significant heterogeneity regarding the underlying airway inflammation, hyperresponsiveness, symptom triggers, and response to anti-inflammatory medications. Most experts agree that asthma is likely to be a syndrome consisting of multiple overlapping disease entities. Unfortunately, we have not yet been successful in translating this increasing understanding of asthma into improved asthma control and reduced morbidity for children. Better methods to assess inflammatory patterns, phenotype characteristics, and likely drug response characteristics are needed to improve outcomes. This review discusses the most recent data regarding the genetics of asthma and practical biomarkers in children, and gives recommendations regarding the best studied and most feasible biomarkers for improving clinical care.

Genetic markers of asthma

Genetic biomarkers are slowly increasing our understanding of how asthma develops, and what genomic loci (along with environmental triggers) are most critical. Early studies searching for asthma susceptibility genes used family-based positional cloning techniques. The first genome-wide screen for asthma is now almost two decades old.[3] Since then genome-wide linkage analyses, and both candidate gene and genome-wide association studies have offered many potential loci that are important in the development of asthma. These studies have directed our attention to the important role of T helper 2 differentiation and action, airway epithelial function, and the innate immune system. Genetic studies have shown us that even the most highly replicated risk genes appear to have only a modest impact on asthma risk. Rather, individual genes likely interact with each other (called epistasis) and the environment to promote asthma. Based on the latest analysis of the literature and considering that physician-diagnosed asthma is a broad array of clinical phenotypes, it is likely that more than 100 different genes influence the onset of asthma. An incomplete list of those genes that have been most replicated and appear most central to children's asthma risk and asthma severity are highlighted in Tables 2 and 3, respectively.

One likely reason that genotype-phenotype associations are difficult to replicate is phenotypic mixing. If association studies do not enforce strict phenotypic characterization (accurate grouping by precise clinical characteristics, including atopy status, age of onset, and bronchial responsiveness) the study will suffer from a type of misclassification bias that will lead to both false discoveries and false negative errors.

A second likely reason for poor replicability is gene-phenotype effect modification by environment or epistatic genes. Important environmental modifiers for asthma can include fetal exposure to maternal smoking, and early postnatal exposure to secondhand smoke, early respiratory virus exposure, and allergens.[4] One important and illustrative example is the interaction between 17q21 variants and early exposure to rhinovirus[5] and tobacco smoke.[6] Caliskan et al who focused specifically on early onset childhood asthma, found that variants in the 17q21 genes (*ORMDL3* and *GSDMB*) were associated with asthma only among children with previous exposure and wheezing with rhinovirus.[5]

Once the clinical diagnosis of asthma is made, several objective biomarkers can be called upon to supplement the child's disease, family and exposure history, and the objective clinical data including physical examination and spirometry. Each biomarker is discussed with particular emphasis on diagnostic utility and treatment management.

Fractional expired nitric oxide

The fraction of expired nitric oxide (FENO) is the most common noninvasive biomarker used for children with asthma. Nitric oxide from expired breath reacts with ozone to produce excited nitrogen dioxide and light energy which, using chemiluminescent analysis, can estimate nitric oxide

Table 2 Genes of particular relevance to childhood asthma risk

Gene	Locus	Biomarker/function	Population	Technique	Citation
DENND1B	1q31.3	Expressed on dendritic cells, interacts with TNFa	North American Whites and Blacks	GWAS	Sleiman[113]
CHI3L1	1q32.1	The protein, YKL-40, is upregulated by 15% among asthmatics. YKL-40 is expressed by macrophages and vascular SMCs and binds to chitins	Hutterites, American Whites, German, Chicago	GWAS	Ober[114]
*IL1RL1/ IL18R1**	2q11.2	Encodes a protein in the Toll-like receptor superfamily/encodes the IL-18 receptor	Icelanders, Europeans, East Asians	GWAS	Gudbjartsson[115]; Moffatt[116]
DPP10	2q14	IL-1 gene cluster	English and German; American Blacks, African Caribbean	Linkage/positional cloning; GWAS	Allen[117]; Mathias[118]
PDE4D	5q12	Phosphodiesterase 4D is a Regulator of ASM contractility	North American children (CAMP)	Association	Himes[119]
IL4	5q31	Encodes IL-4	North American Whites	Association	Basehore[120]
PCDH1	5q31-q33	Protocadherin 1 encodes an adhesion molecule on alveolar macrophages and epithelial cells	Dutch; Netherlands, UK, USA	Linkage/positional cloning	Koppelman[121]
*HLA-DR/ DQ**	6p21	MHC class II loci	American Whites	GWAS	Li[122]; Moffatt[116]
GPRA	7p	A G-protein coupled receptor	Finnish, Canadian, Australian	Linkage/positional cloning	Daniels[3]; Laitinen[123]; Leaves[124]
*IL33**	9p24	Encodes IL-33, belongs to the IL-1 superfamily of proteins	Europeans	GWAS	Moffatt[116]
ORMDL3 cluster*	17q21	Encodes transmembrane proteins anchored in the ER, of unknown function	European, Asian, Hispanics	Association, GWAS	Moffatt[116]; Caliskan[5]
ADAM33	20p13	A disintegrin and metallopeptidase domain 33 membrane-bound metalloprotease protein	White sibling pairs from North America and UK	Linkage/positional cloning	Van Eerdewegh[125]

Abbreviations: TNFa, tumor necrosis factor alpha; GWAS, genome-wide association study; SMC, smooth muscle cell; ASM, airway smooth muscle; CAMP, children's asthma management program; MHC, major histocompatibility complex; IL, interleukin; ER, endoplasmic reticulum.
Note: *associated with early onset asthma.

concentration. Both online and offline methods measure FENO in parts per billion. Offline measurement involves participant exhalation into an impermeable reservoir that is analyzed for nitric oxide content. Online measurement uses exhalation directly into a handheld portable device. Most children can reproducibly perform a valid 6-second maneuver required for FENO measurement,[7,8] 64% and 93% of 6 and 8 year olds, respectively, were able to perform reproducible FENO measures. The handheld Niox Mino® (Aerocrine AB, Solna, Sweden) can be successfully used in children down to 4 years of age.[9]

In the pediatric age range, FENO is most closely associated with allergic and eosinophilic airway inflammation, and likely reflects the contribution of nitric oxide metabolism in airway inflammation.[10] Other factors associate with FENO that are not directly related to asthma. FENO levels positively correlate with male gender,[11] older age, lower body mass index, allergic sensitization,[12] pollen[13] or air pollution exposure,[14] sputum eosinophils,[15,16] persistent airway inflammation responsive to inhaled corticosteroids,[17,18] Asian ethnicity,[19] morning time of day,[20] gene polymorphisms in the nitric oxide pathway[21] and acute stress. Smoking or exposure to secondhand smoke also modestly reduces FENO.[22–24] Importantly, FENO does not consistently correlate with lung function[25,26] or bronchial hyperreactivity.[27] FENO does not closely or consistently reflect or predict asthma symptoms.[20]

Practical uses

FENO is one of the easiest, most feasible, and noninvasive biomarkers available, allowing point-of-care feedback in the clinical setting. In asthmatic children with elevated FENO, levels tend to drop following treatment with inhaled corticosteroids. FENO-driven monitoring and medication adjustment have been studied with the hypothesis that serial FENO measures may allow safer weaning of controller therapy and improved control. In adults, FENO-guided management has been shown to lead to modest improve-

Table 3 Genes important to childhood asthma severity or drug response

Gene	Locus	Protein	Association effect (pathogenesis, severity, treatment)	Citation
SPATS2L	2q33	Unknown function, expressed in many cell types, involved in ribosomal biogenesis	Association study, Related to bronchodilator responsiveness	Himes[126]
ADBR2	5q31	Beta2 adrenoreceptor; Transmembrane domain receptor on ASM and inflammatory cells	Arg16 variant is associated with improved response to Beta2 agonism	Israel[127]; Lima[128]; Martinez[129]
IL4	5q31	Encodes interleukin-4	Associated with reduced FEV1, North American Whites	Burchard[130]
CD14	5q31	Encodes CD14 receptor for lipopolysaccharide	Associated with asthma severity with modification from SHS; associated with severity; Hispanic/ Latino, North American Whites, Australian	Choudhry[131]; Martin[132]
T	6q27	Encodes a mesodermal developmental transcription factor with DNA binding capability.	Association study, Response to ICS	Tantisira[133]
GLCCI1	7p21.3	Glucocorticoid inducible gene	Association study, Response to ICS	Tantisira[109]
ALOX5	10q11.2	5-lipoxygenase	Sp1 binding motif repeat polymorphism gentoype associated with improved response to ALOX5 inhibitor; worse lung function and asthma control	Drazen[134]; Mougey[135]
CRHR1	17q12-22	Intronic region of the corticotrophin releasing hormone receptor 1 gene	Association study, Response to ICS	Tantisira[136]
ORMDL3 cluster*	17q21	Encodes transmembrane proteins anchored in the ER, of unknown function	Association with exacerbations and poor asthma control on controller medication, Europeans	Bisgaard[137]; Tavendale[138]
ADAM33	20p13	A disintegrin and metallopeptidase domain 33 membrane-bound	ADAM33 increased in severe versus mild asthma or no asthma, English	Foley[139]

Abbreviations: Arg, Arginine; ASM, airway smooth muscle; ER, endoplasmic reticulum; FEV1, forced expiratory volume in 1 second; SHS, secondhand smoke, ICS, inhaled corticosteroids; LTM, leukotriene modifier.

ment in asthma control despite reductions in inhaled corticosteroid dose.[28] The utility of FENO-driven care in children has not been robustly supported by the current data and may even lead to reduced weaning of controller steroids.[29] A recent systematic review of the current evidence concluded that the routine use of FENO-based management could not be recommended.[30] Despite this, FENO-guided management may be helpful in select patients. Handheld on-line FENO devices exist and have been shown to be feasible for daily home use.[20] Among children with atopy and asthma, fluctuation patterns in daily FENO may predict future loss of asthma control.[18,31] However, more data are needed to establish concrete practical uses of FENO in predicting poor asthma control and exacerbations. FENO may be useful in identifying patients who will respond to inhaled corticosteroids. FENO was higher 2 and 4 weeks following asthma exacerbation among patients requiring more post-exacerbation rescue treatment; and among patients not prescribed post-exacerbation inhaled corticosteroid.[32] An obvious and very practical application of any biomarker in young asthmatics is the possible predictive value for later persistent wheezing. Unfortunately, FENO in toddlers with asthma symptoms added only modest improvements to the predictive abilities for later physician-diagnosed asthma during school age.[33,34] A summary of practical uses for FENO is included in Table 4.

Limitations

Several important limitations exist with FENO that must be understood to maximize its practicality. Since FENO is not

Table 4 Uses of FENO in the clinic

When FENO may be helpful

1. Identifying patients who are likely to respond to inhaled corticosteroids
2. Identifying patients with allergic and eosinophilic airway inflammation
3. Establishing diagnosis of persistent asthma among patients with chronic asthma-like symptoms
4. Guiding asthma medication management in select patients with allergic/eosinophilic asthma demonstrating high pre-treatment FENO levels. FENO may help as supplemental data for determining titration of steroid dosing and adherence[140]
5. Adding supplemental predictive data to toddlers/young school children with asthma symptoms (will child develop persistent asthma?)

When FENO is not as helpful or should be assessed with caution

1. Determining who currently has asthma and who does not have asthma
2. Detecting poor asthma control[140,141]

Abbreviation: FENO, fractional exhaled nitric oxide.

specific to asthma and may be influenced by other factors, it is not surprising that there exists considerable overlap in FENO between asthmatics and non-asthmatics. In addition, FENO is generally not a good marker of current or future asthma symptoms and is not associated with steroid-related improvements in lung function following an asthma exacerbation.[32] Optimal cutpoints to differentiate asthma and nonasthma respiratory symptoms, and controller adherence versus nonadherence,[27] both remain elusive. Additionally, considerable overlap exists between asthma patients when on or off daily inhaled steroids.[27] No FENO cutpoint appears to have both acceptable sensitivity and specificity.[35]

A recent National Institutes of Health committee on biomarkers recommended that FENO be a supplemental (rather than a core) marker for the characterization of asthma cohorts in clinical trials and observational studies.[36] Areas that need further exploration include the relationships between FENO and airway remodeling, asthma control, and phenotype.

Total and allergen-specific IgE

The atopy status of a child with asthma is a very important determination for clinicians to make for both prognostication and management. Asthma with comorbid allergen sensitization typically constitutes a distinct clinical phenotype from other common pediatric phenotypes including intermittent viral-induced asthma, exercise-induced asthma, or late onset obesity-related asthma. Total serum Immunoglobulin E (IgE) generally and allergen specific IgE specifically are accepted biomarkers for atopic asthma in the clinical setting of asthma symptoms. The Phadiatop™ (Pharmacia and Upjohn, Uppsala, Sweden) multiallergen screen was recently declared a core biomarker for the description of asthma populations participating in research studies.[36] It characterizes someone as atopic but does not specify which of the most common indoor and outdoor allergens are likely present. The Phadiatop assay uses the ImmunoCAP (Childhood Allergen Profile) technology with a solid-phase immunoassay for serum specific IgE. The proprietary mixture reports to include the most relevant inhalant allergens coupled with ImmunoCAP.

Total IgE has been associated with asthma[37,38] and is highly age-dependent. Compared with nonatopic infants, atopic infants have an earlier rise and peak in serum IgE. Total serum IgE reaches adult levels by age 10–15 years and declines from the second decade. Still, there is considerable overlap in IgE levels between atopic and nonatopics. The presence of allergen-specific IgE in the setting of asthma and allergy symptoms defines an individual as having atopic asthma.[39,40] Secondly, allergen-specific IgE can detect a likely asthma and allergy trigger that can become a focus of prevention efforts. There is a direct relationship between level of serum IgE and likelihood of wheeze and likelihood of reduced lung function. IgE serum levels to mite, cat, and dog at age 3 years are associated with an increased risk of wheeze at age 5 years, while a similar increase in wheeze was not associated with local size of skin test wheal.[40]

The multiallergen screen for aeroallergens (Phadiotop) plus the food allergen mix (f × 5) has generally been more effective for the characterization of asthma than measuring individual allergens.[41,42] When the Phadiotop and f × 5 are used in combination, they display a 97.4% positive predictive value for any suspected allergic disease, eg, asthma, allergic rhinitis, eczema, food allergy. Young children with episodic wheeze and significant elevation in the multiallergen screen are at higher risk for persistent wheeze that will continue into adolescence. These children will require close monitoring and a thoughtful asthma and allergy plan in place to reduce morbidity.

Urinary leukotrienes

Leukotrienes are formed via arachidonic acid metabolism through the 5-lipoxygenase pathway. Phospholipase A_2 activates the release of arachidonic acid from inflammatory cell-membrane phospholipids which is converted to leukotriene A_4 by membrane-bound 5-lipoxygenase. The latter is activated by the action of 5-lipoxygenase-activating protein.[43] Leukotriene A_4 hydrolase converts leukotriene A_4 to leukotriene B_4, or leukotriene C_4 synthase conjugates leukotriene A_4 with reduced glutathione to form leukotriene C_4.[43] Leukotriene B_4 and leukotriene C_4 are transported to the extracellular space[44,45] where leukotriene C_4 is converted to leukotriene D_4 by γ-glutamyltransferase.[46] Leukotriene E_4 is formed from leukotriene D_4 by dipeptidase.[47] Biosynthesis of cysteinyl leukotrienes (leukotrienes C_4, D_4, and E_4) occurs primarily in eosinophils, basophils, and mast cells, whereas leukotriene B_4 is found largely in neutrophils, macrophages, and dendritic cells.[43] The cysteinyl leukotrienes bind to and activate cys-leukotriene 1 and cys-leukotriene 2 receptors, causing bronchoconstriction, airway hyperresponsiveness and smooth muscle hypertrophy, mucus hypersecretion and mucosal edema, and influx of eosinophils into airway tissue.[48,49] The most commonly used leukotriene modifier (montelukast) blocks the cys-leukotriene 1 receptor.

Formation and metabolism of cysteinyl leukotrienes occurs rapidly. Leukotriene A_4 exists only as an unstable intermediate and leukotriene C_4 is rapidly (in minutes) converted to leukotriene D_4.[50] The latter is converted to

leukotriene E_4 over approximately 30 minutes, and within approximately 4 hours, 5%–16% of leukotriene E_4 is excreted in the urine.[50–52] As the most stable of the cysteinyl leukotrienes, urinary leukotriene E_4 can provide a sensitive noninvasive measure of whole-body cysteinyl leukotriene production.[53] Urinary leukotriene E_4 levels (pg/mg creatinine) can be measured using an enzyme-linked immunosorbent assay commercial kit (Cayman Chemical Company, Ann Arbor, MI, USA, lower limit 25 pg/mL; Glory Science Co, Ltd, Del Rio, TX, USA, lower limit 5 pg/mL) or liquid chromatography-tandem mass spectrometry.[54]

Urinary leukotrienes as a biomarker for disease or disease severity

Significantly higher urinary leukotriene levels (two-fold) have been noted in patients with aspirin-sensitive asthma compared with those with aspirin-insensitive asthma or patients without asthma.[53,55] Levels have not been shown to be consistently elevated in patients with stable asthma compared with controls.[53,55] Aspirin-sensitive asthma with polyposis may reflect a unique phenotype associated with upregulated leukotriene biosynthesis because urinary leukotriene levels in these patients are higher than in patients with asthma and aspirin sensitivity but without polyposis.[53]

Children with sickle cell disease and asthma may also represent a unique disease phenotype with increased leukotriene production. Children with sickle cell disease and asthma have significantly greater urinary leukotriene levels compared with those with only asthma (151 pg/mg versus 61 pg/mg creatinine, respectively)[56] although there was no difference noted in adults with sickle cell disease with and without asthma.[57]

Inconsistencies between disease groups may reflect the variability associated with age, because children may have higher levels than adults, or the methodology selected for detection.[36,53] For this reason, intraindividual variation may be more clinically relevant than interindividual differences. In this context, patients with nocturnal asthma had urinary leukotriene levels which were higher during the 3 am to 6 am period than from 3 pm to 6 pm.[58] Similarly, increases in nocturnal symptoms coincided with falls in peak expiratory flow and increases in urinary leukotriene over time in young adults with asthma but only in those patients with nocturnal symptoms.[59] Urinary leukotriene increases have also been associated with increased use of albuterol in children over time.[53] Other studies have shown that urinary leukotriene increases during an acute asthma exacerbation and levels fall as symptoms improve.[53]

Urinary leukotrienes as a biomarker for drug response

In several pediatric studies of children with asthma, higher urinary leukotriene levels have predicted the clinical response to montelukast, a cys-leukotriene 1 receptor antagonist. Szefler et al found that young children (under 10 years old) with creatinine levels of 100 pg/mg or more were more likely to respond by an improvement in forced expiratory volume in one second (FEV_1) of ≥7.5%.[60] In another study in children, the ratio of urinary leukotrienes to exhaled nitric oxide was positively associated with response to montelukast and negatively associated with response to fluticasone propionate, an inhaled corticosteroid.[61] In adolescents and adults with asthma, responders (improvements of 20% in symptom scores and albuterol use and 10% improvement in FEV_1) to montelukast had significantly higher urinary leukotriene levels of 225 pg/mg creatinine compared with 175 pg/mg creatinine in nonresponders.[62] The National Institutes of Health committee on biomarkers strongly recommended monitoring urinary leukotriene in any study involving manipulation of the eicosanoid pathway.[36] The committee recommended its use in studies that characterize asthma phenotypes, particularly aspirin-sensitive, adult-onset, and eosinophilic asthma.[36]

Genetic variants in the leukotriene pathway and response to treatment

Genes in the leukotriene synthesis pathway are highly polymorphic and many studies have evaluated associations between variants and response to treatment.[63] The *ALOX5* gene located on 10q11.21 encodes ALOX5, a key enzyme in the synthesis of cysteinyl leukotrienes.[64] Early studies identified addition and deletion variants (nucleotide sequences of 5′GGGCGG3′ repeated in a tandem cluster where wild-type n = 5 and variant n≠5), in the core promoter (factor Sp-1 binding motif) of *ALOX5*, the gene encoding 5-lipoxygenase, that were associated with diminished promoter-reporter activity in tissue culture.[65] Because this is the first enzyme in the leukotriene synthesis pathway, it would be expected to exert a possibly larger influence on response than other downstream pathway genes. Indeed, early studies found that patients with asthma carrying at least one wild-type allele (five repeats) had improved outcomes after taking a 5-lipoxygenase inhibitor or montelukast compared with patients homozygous for the variant allele.[66,67] Lima et al and Klotsman et al also found an association, but in these studies the carriers of the variant allele had an improved response to montelukast.[68,69] A recent study examined *ALOX5* polymorphisms and urinary

leukotriene concentrations and found that leukotriene levels were significantly higher in children with asthma who were homozygous for the variant allele, substantiating the work by Lima and Klotsman and suggesting that the presence of the variant allele may upregulate cysteinyl leukotriene production, leading to an enhanced response to leukotriene modifiers in those studies.[54]

Polymorphisms in other genes, such as *CYSLTR1*, *LTA4H*, *LTC4S*, and transporter genes, have also been studied for response to drug therapy, but few studies have replicated these results[68,70] (Table 5). In the largest candidate gene pharmacogenomic study, Lima et al genotyped 28 polymorphisms from *ALOX5*, *cysLTR1*, *LTA4H*, *LTC4S*, and *ABCC1* (was *MRP1*), and associations with a beneficial response to montelukast were identified for *ABCC1* rs119774 and *ALOX5* rs2115819 for FEV_1 response, and *LTA4H* rs2660845 and *LTC4S* A-444C (C carriers) for reduced risk of exacerbation.[71] This study was repeated with zileuton and the findings for *ALOX5* rs2115819 and *ABCC1* rs119774 were replicated.[72] In another study of six pathway genes (*ALOX5*, *LTC4S*, *CYSLTR1*, *CYSLTR2*, *ALOX5AP*, *PLA2G4A*) with 15 polymorphisms, improvements in lung function in patients taking montelukast were observed for variants in *ALOX5*, *ALOX5* addition/deletion, *CYSLTR1*, and *CYSLTR2*, although none replicated the findings by Lima et al.[71] In the studies by Lima et al and Klotsman et al, haplotype analysis yielded additional associations with response to montelukast.[71,73] In a study of zafirlukast, a cys-leukotriene-1 receptor antagonist, patients with asthma who were carriers of the C allele in *LTC4S* A-444C had greater improvements in lung function and produced more leukotriene C_4 from eosinophils.[74] Similarly, in a Japanese population of patients with asthma treated with pranlukast (a cys-leukotriene-1 receptor antagonist available outside the United States), carriers of the *LTC4S* A-444C C allele had a better lung function response compared with AA homozygotes.[75] In another study of montelukast, reduction in FENO was greater (albeit not statistically significant) in carriers of the *LTC4S* A-444C C allele, although lack of significance may have been due to a small sample size (n = 12).[76] G allele carriers of the *LTA4H* rs2660845 polymorphism have also been shown to have a poorer lung function response to montelukast in Japanese patients, which supports the finding for this same polymorphism by Lima et al.[71,77] Replication of findings among these studies suggests that variants in *ALOX5* (addition/deletion polymorphism), *ALOX5* (rs2115819), *ABCC1* (rs119774), *LTC4S* A-444C, and *LTA4H* (rs2660845) genes may associate with response to leukotriene modifiers.

Currently available leukotriene modifiers are administered orally, so variation in therapeutic effect may be caused by polymorphisms in genes regulating uptake and transport from the gut and metabolism in the liver. Montelukast is metabolized primarily by cytochrome P450 (CYP)2C8 in the liver (72% of oxidative metabolism) with smaller contributions from CYP3A4 (16%) and CYP2C9 (12%).[78] The gene encoding the hepatic CYP2C8 enzyme is highly polymorphic but no pharmacogenomic studies have been performed.

Table 5 Pharmacogenomic association studies of polymorphisms in the leukotriene pathway

Gene	Polymorphism	Drug	Associated outcome	Reference
ALOX5	rs59439148[1]	Montelukast	Exacerbation rate	Lima[71]
ALOX5	rs59439148[1]	5-LO inhibitor	Lung function	Drazen[134]
ALOX5	rs59439148[1]	Montelukast	Lung function; exacerbation rate; beta agonist use	Telleria[142]
ALOX5	rs2115819	Montelukast	Lung function	Lima[71]
ALOX5	rs2115819	Zileuton	Lung function	Tantisira[72]
ALOX5	rs4987105	Montelukast	Lung function	Klotsman[73]
ALOX5	rs4986832	Montelukast	Lung function	Klotsman[73]
CYSLTR2	rs91227	Montelukast	Lung function	Klotsman[73]
CYSLTR2	rs912278	Montelukast	Lung function	Klotsman[73]
LTC4	A-444C promoter	Montelukast	Lung function	Asano[75]
LTC4	A-444C promoter	Zafirlukast	Lung function	Sampson[74]
LTC4	A-444C promoter	Montelukast	FENO	Whelan[76]
LTA4H	rs2660845	Montelukast	Lung function	Kotani[77]
LTA4H	rs2660845	Montelukast	Exacerbation rate	Lima[71]
ABCC1 (MRP1)	rs119775	Montelukast	Lung function	Lima[71]
ABCC1 (MRP1)	rs119775	Zileuton	Lung function	Tantisira[72]
SLCO2B1	rs12422149	Montelukast	Plasma concentrations; asthma control	Mougey[79]

Note: [1]rs59439148 is an Sp1 tandem promoter repeat polymorphism.
Abbreviations: LO, lipoxygenase; FENO, fractional exhaled nitric oxide; Sp1, serum protein 1.

An elegant study by Mougey et al confirmed that montelukast undergoes carrier-mediated uptake from the gut by OAT2B1 and OAT1A2 (encoded by *SLCO2B1* and *SLCO1A2*, respectively).[79] A variant in *SLCO2B1* (rs12422149) alters the protein coding of OAT2B1 and associates with reduced plasma concentrations of montelukast and worse asthma control.[79] In summary, urinary leukotriene levels are a sensitive biomarker of changes in asthma severity and response to drug treatment, and can be collected and measured in children who are too young to provide other outcome measures in a dependable manner, such as pulmonary function, symptom scores, and albuterol use related to subjective symptom assessment. Additional research is needed to confirm findings from previous studies of the *ALOX5*, *LTC4S* A-444C, *LTA4H*, *CYSLTR1*, *CYSLTR2*, *ABCC1,* and *SLCO2B1* polymorphisms before they can be reliably used to predict treatment response. In the future, ideally, leukotriene concentrations would be measured in the lung where they are biologically relevant for causing symptoms of asthma. Exhaled breath is a gaseous mixture of volatile compounds and a liquid phase collected as a condensate. The collection of exhaled breath condensate is technically simple and can be performed by children as young as 4 years and involves breathing in while holding a mouthpiece between the lips for approximately 10 minutes. Exhaled breath condensate can be quantitatively analyzed for inflammatory mediators and related constituents (8-isoprostane, nitrates, nitrites, nitrotyrosine, tumor necrosis factor-alpha) and pH. A recent review of the exhaled breath condensate literature in pediatric asthma found that the cysteinyl leukotrienes were measurable in exhaled breath condensate, that increased levels were found in children with asthma compared with healthy controls, and higher levels were associated with increased asthma severity.[80] However, condenser coatings and sample handling (freeze-thaw) can significantly affect biomarker concentrations.[81,82] The ECoScreen (Jaeger, Wuerzburg Germany) has been shown to measure higher levels of cysteinyl leukotrienes than the R-Tube (Respiratory Research, Inc, Charlottesville, VA, USA), possibly reflecting the impact of condenser coatings or other factors on sample stability.[82–84] The R-Tube is currently widely used in large clinical trials for its lower cost and ease of sample collection compared with other devices, but at present is not suitable for measurement of cysteinyl leukotrienes.

Biomarkers to assess secondhand tobacco smoke exposure

Despite years of tobacco education, nearly 20% of US adults continue to smoke on a daily basis.[85] Secondhand tobacco smoke is the primary indoor pollutant for most children, with more than 40 million US children exposed each year.[86] Recent data suggest that more than half of children in the US are exposed at least intermittently to secondhand tobacco smoke.[87] Secondhand tobacco smoke exposure increases the risk for reduced lung growth, sudden infant death syndrome, pneumonia, bronchitis, ear infections, and asthma. A recent large meta-analysis reported that among infants exposed to secondhand tobacco smoke, the odds of incident wheeze was increased 70% compared with those not exposed (95% confidence interval 1.24–2.35).[88] In children already with asthma, exposure to secondhand tobacco smoke can make daily asthma control very difficult to achieve and can induce severe asthma attacks.[89,90] Secondhand smoke (SHS) contains over 250 toxic and carcinogenic chemicals (including arsenic, formaldehyde, and cyanide), with only small amounts needed to impose lung damage, respiratory infections, oxidant damage, airway reactivity, and asthma-like symptoms.[91–95] The data linking SHS with childhood asthma are considerable.[86,96–98] Asthmatic children exposed to SHS are particularly at risk for accelerated loss of lung function and worsening disease severity,[91,93,99–102] and may be at increased risk for death.[103,104] The mechanisms underlying passive smoking-related morbidity in children with asthma are not completely understood but may involve several mechanisms including greater acquisition of respiratory viruses which serve as asthma triggers, or upregulation of the leukotriene pathway, given that active smoking increases the production of cysteinyl leukotrienes and leukotriene B4.[6–9]

A reliable biomarker for the detection of recent exposure to secondhand tobacco smoke would be clinically helpful in certain scenarios. Secondhand tobacco smoke can be measured objectively from directly sampling nicotine metabolites or tobacco-specific carcinogens. Blood samples provide the most accurate assessment of exposure level occurring in the previous 72 hours. Cotinine, a primary metabolite of nicotine, can be measured in the blood, or less invasively measured in saliva, hair, and urine by reference laboratories.[105,106] Cotinine has a half-life of roughly 16 hours and is completely eliminated from the body within about 3 days.[107] Cotinine results are subject to short-term variations in exposure to secondhand tobacco smoke and significant within-subject variability. An alternative biomarker is 4-(methylnitrosoamino)-(3-pyridyl)1-butanol (NNAL), which is a tobacco-specific carcinogen and can be measured in the urine. NNAL is usually not detectable in nonsmokers and has a considerably longer half-life (10–16 days)[108] compared with cotinine. The longer half-life of NNAL may eventually make it more useful in detecting

Table 6 When urinary or serum cotinine may be useful*

- To assess the effectiveness of home SHS reduction measures (detection of thirdhand smoke[143])
- To assist motivated caregivers in their tobacco cessation efforts
- To determine if SHS may be present in the context of poor asthma control
  - To assess if children are being exposed during visitations
  - To assess if children are being exposed in automobiles

Notes: *Detection of secondhand smoke exposure in the proper clinical context may assist the clinician and caregiver to have a better idea of how much intermittent exposure may be playing a role in poor asthma control. Knowledge gained from testing needs to be considered carefully against perceived issues of privacy.
Abbreviation: SHS, secondhand smoke.

exposure among children who have an intermittent exposure pattern. Currently, more research is needed in validating both biomarkers to improve sensitivity and specificity. Despite its infrequent use, urinary or serum cotinine measurement may be clinically helpful in select circumstances (Table 6) when a caregiver or provider is unsure of the degree of secondhand tobacco smoke to which a child is exposed.

Conclusion

How can this article help foster personalized care for asthma? Admittedly, the field of personalized medicine in the care of children with asthma is still in its infancy. Progress has been made in identifying pharmacogenetic targets for inhaled corticosteroids[109] and leukotriene modifier[71] responses. We are not yet able to apply stratified asthma treatments based on genetic information, although this time may be fast approaching. The few biomarkers available to assist clinicians relate to indicators of the underlying molecular mechanisms driving asthma. FENO and serum IgE (including allergen-specific IgE), when evaluated in the context of recurrent asthma symptoms, are general predictors of allergic airway inflammation and help to guide clinicians in understanding the likely disease course and response to the most common asthma controllers. Biomarker assays for secondhand tobacco smoke exposure and cysteinyl leukotrienes are both promising areas of study that can help personalize management, not just for pharmacologic management but also education and prevention efforts.

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Disclosure

The authors report no conflicts of interest in this work.

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