

Original article

ALOX5 promoter genotype, asthma severity and LTC₄ production by eosinophils

Background: The number of Sp1–Egr1 binding tandem repeats at the ALOX5 promoter influences gene transcription and may modify the response to anti-leukotriene treatment. The relationship of ALOX5 variants to asthma severity and leukotriene production by eosinophils is unknown.

Objective: To characterize ALOX5 mRNA expression and cysteinyl-leukotriene production by eosinophils from individuals bearing ALOX5 promoter deletion variants and their association with the severity of childhood asthma.

Methods: Eosinophils from adult asthmatics bearing only variant alleles (with other than five tandem repeats on both chromosomes, non5/non5) or no variant alleles (5/5) were cultured *in vitro* and ALOX5 expression and leukotriene secretion were measured. A total of 621 children with mild or moderate-severe asthma were genotyped at the ALOX5 core promoter.

Results: Asthmatics with non5/non5 genotype expressed less ALOX5 mRNA and produced less LTC₄ into culture supernatants than 5/5 individuals (6.4 ± 2.0 and 20.0 ± 5.0 pg/ml, $n = 5$; $P < 0.05$). More asthmatic children bearing non5/non5 genotype had moderate-severe asthma than children with the 5/5 genotype (5.3% vs. 1.4%, $P = 0.008$). Multivariate logistic regression identified ALOX5 promoter genotype as a significant predictor of disease severity (OR = 3.647, 95% CI: 1.146–11.608, $P = 0.03$). Consistent with these findings, children bearing the non5/non5 genotype had greater bronchomotor response to exercise as measured by the maximum fall after exercise and the area under the exercise curve ($P < 0.05$ for both).

Conclusion: Our results suggest that children who express the asthma phenotype despite having a genetic variant that impairs their ability to express ALOX5 have more severe disease and thus are more likely to have asthma symptoms.

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Cysteinyl-leukotrienes are important mediators of asthma. They induce bronchoconstriction (1–7), tissue edema (8), and eosinophil migration (9, 10) and stimulate airway secretions (11, 12). Furthermore, inhibition of leukotriene activity is associated with clinical benefits (13, 14). There is some evidence that the therapeutic response to leukotriene modifiers is a function of the genotype of the gene encoding 5-lipoxygenase (ALOX5) genotype (15), but the effects of the ALOX5 genetic variants on leukotriene production by effector cells of the allergic response, such as eosinophils, and the relation of these variants to asthma severity are unknown.

The transcription of ALOX5 is governed partly by a G + C-rich promoter region containing insertion/deletion genetic variants (16, 17). The most common form contains five zinc finger (Sp1/Egr-1)-binding motifs in

tandem. Mutations characterized by the addition or deletion of these motifs (i.e. –GGGCGG–) have been discovered (18). In *in-vitro* models, transactivation of the ALOX5 promoter by the transcription factor Sp1/Egr-1 depends on the number of 6-bp tandem-repeat motifs (16, 18). That these variants have clinically detectable effects is evident from a study showing that asthmatics with deletion forms of ALOX5 do not respond as well to treatment with a 5-lipoxygenase inhibitor as individuals bearing two wild-type alleles (15).

A second enzyme in the biosynthesis of cysteinyl leukotrienes is leukotriene C₄ (LTC₄) synthase. A functional transversion in the promoter of LTC₄ synthase gene (A444C) was described (19), and the C allele was shown to increase the relative risk of aspirin-induced asthma in a Polish (20) but not in a Korean population

(21). Within the Korean population, however, a significant association was observed between aspirin-intolerant asthma and a certain ALOX5 haplotype (21). In a Caucasian population, on the other hand, Syers *et al.* showed that the number of tandem repeats in the ALOX5 promoter is not associated with asthma or asthma-related phenotypes (22).

Despite these findings, the effects of these variants on asthma severity and leukotriene production by eosinophils are unknown. We, therefore, sought to define the effects of ALOX5 variants on mRNA expression and cysteinyl-leukotriene production by eosinophils from individuals bearing ALOX5 promoter deletional variants and test the hypothesis that asthmatic children bearing ALOX5 or LTC₄-synthase promoter variants have more severe disease than those bearing wild-type alleles.

Material and methods

Subjects

The volume of blood required to obtain sufficient eosinophils to measure ALOX5 mRNA and LTC₄ *in-vitro* dictated that this study be performed in adults. Adult subjects met the American Thoracic Society definition of asthma (23) and had improvement in FEV₁ of 15% or greater after inhalation of a short-acting beta-agonist or had a metacholine PC20 < 8 mg/ml. Each subject provided written informed consent.

Children with asthma aged 6–18 years were recruited from the Pediatric Allergy and Asthma Unit, Hacettepe University School of Medicine, Ankara, Turkey, between October 2002 and September 2003. Criteria for asthma diagnosis and severity have been detailed previously (24) and were in strict accordance with GINA Guidelines (25).

Skin testing employed a battery of 12 aeroallergens in adults and eight food and 30 aeroallergens (Allergopharma, Hamburg, Germany) in children. Reactions with indurations > 3 mm larger than negative control were considered positive. DNA was extracted from whole blood (26).

Samples were coded, and the table of cross-references was destroyed. Separate informed consent was obtained before study in a subset of subjects bearing informative genotypes. The individuals who determined the phenotype of the cells or subjects being studied were unaware of the genotype.

All study procedures were done in accordance with protocols previously approved by the Ethics Committee of Hacettepe University. All parents provided written informed consent and children gave assent.

Study design

The *ex-vivo* eosinophil study was designed as a prospective, blinded, genetically stratified parallel-group study with mRNA expression and LTC₄ secretion by eosinophils as the primary outcome parameters. The results for individuals homozygous for the ALOX5 wild-type 5-tandem-repeat allele was compared with those from individuals bearing variant alleles on both chromosomes. This study was performed in duplicate, with its replication being stratified by race.

Factors likely to be effective in determining the severity of asthma, including ALOX5 genotype, were identified by logistic

regression analyses. The degree of airway contractile responses to exercise, an objective measure of asthma severity, was compared in individuals bearing the ALOX5 genotypes described above.

Study procedures

ALOX5 promoter genotyping. Samples were genotyped by ABI PRISM 3100 capillary technology using a previously described protocol (15, 18). PCR was performed with fluorescence-labeled (6 FAM) primers: 5'-ACA GAC ACC TCG CTG AGG AGA GAC-3'; and 5'-GAG CAG CGA GCG CCG GGA GCC T-3'. PCR reactions were performed in a volume of 50 µl containing 200 ng of genomic DNA, PCR buffer with MgCl₂ (Boehringer Ingelheim, Mannheim, Germany), 1 mM MgCl₂, 5% DMSO, 200 µM dATP, dTTP, dCTP, and 7-deaza-dGTP, 20 pmol of each primer, and 3 U of Taq polymerase. The reaction was performed for 40 cycles of 94°C for 15 s, 60°C for 23 s, and 72°C for 5s, followed by a chain elongation step at 72°C for 5 min. Genotyping was confirmed by measuring the length of the PCR product on a 4% Agarose + NuSieve agarose (1 : 1) gel. ALOX5 alleles were designated by the number of –GGGCGG– repeats; the wild-type genotype was designated 5/5 and those without wild-type alleles as non5/non5.

LTC₄ promoter genotyping. Genotyping of the LTC₄ synthase promoter region was performed as described (27). Ten samples from each genotype were confirmed by sequencing.

Purification of eosinophils

Blood was obtained from asthmatic individuals with alternative ALOX5 genotypes. Granulocytes were isolated by Histopaque separation with 1077 and 1119 gradients followed by 1077 gradient (Sigma, St Louis, MO). Erythrocytes were lysed by hypotonic lysis. Eosinophils were separated from polymorphonuclear leukocytes by CD16 antibody–negative magnetic selection (Dynaltech, Oslo, Norway). More than 97% of the cells isolated had vital-staining characteristics of eosinophils.

Culture of eosinophils

Eosinophils were resuspended in RPMI-1640 medium containing 50 mM L-serine supplemented with 10% FBS and streptomycin-100 U/ml, penicillin G 100 µg/ml, at a concentration of 1 × 10⁶/ml and divided into 1-ml aliquots. Aliquots were untreated or treated with 10 µM zileuton. Cells were then incubated for 24 h at 37°C in 5% CO₂ and centrifuged; supernatants were collected and stored at –80°C until measurement. RNA was isolated from the cell pellets with the Qiagen RNeasy kit (26).

Comparison of ALOX5 mRNA expression

RNA was reverse-transcribed (Retro Script, Ambion) and cDNA amplified with primers left 5'-ATCAGGACGTTTCACGGCCGAG-3', right 5'-CCAGGAACAGCTCGTTTTCCTG-3' under the following conditions: 94°C 1 min, 60°C 1 min, and 72°C 1.5 min for 35 cycles. Expression of the ALOX5 gene was compared with that of the constitutively-expressed cyclophilin-A gene using the primers left 5'-AGGTCCCAAAGACAGCAGAA-3', right 5'-TGTCACAGTCAGCAATGGT-3' under the following conditions: 94°C 45 s, 60°C 45 s, and 72°C 2 min for 25 cycles. PCR product was electrophoresed on 1.5% agarose gel.

Mediator measurements

Quantification of LTC₄ in cell-culture supernatants was performed with an enzyme immunoassay kit specific for LTC₄ (Cayman Chemicals, Ann Arbor, MI, USA). Samples were run in duplicate. The coefficient of variation for repeated measurement was less than 15%. IgE levels were measured in duplicate with Uni-Cap (Pharmacia, Kalamazoo, MI). Eosinophil counts were determined from Coulter Counter measurements.

Exercise testing

Exercise testing was conducted according to a previously described protocol (28). All medications were discontinued in accordance with American Thoracic Society guidelines (29) and inhaled corticosteroids were withheld for at least 15 days before the exercise challenge. A reduction in FEV₁ of at least 10% of the pre-exercise value was considered abnormal (30).

To determine whether exercise responses are a function of genotype, we compared the maximal decrease in FEV₁ and the area under the curve (AUC) for FEV₁ during the 20 min after the exercise in individuals bearing variant alleles with those bearing wild-type alleles on both chromosomes. Exercise challenge was stopped for children with a decrease in FEV₁ > 20% of baseline and in those with asthma symptoms, and a β -agonist was administered and the last FEV₁ measurement before rescue therapy was carried forward. The signed AUC was obtained by connecting the sample points by straight lines and was calculated by the formula $(\frac{1}{2}h_0 + h_5 + h_{10} + h_{15} + \frac{1}{2}h_{20}) \times (5 \text{ min})$, where h_t is the value at time t . Values that exceeded baseline FEV₁ were considered as if the FEV₁ had returned to baseline value (31).

Statistical analyses

Statistical analyses were performed with Sigma Stat 2.0 and SPSS 11.5 for Windows. Hardy-Weinberg equilibrium was tested with chi-square statistic. The data in both the *ex-vivo* eosinophil and exercise studies were tested for assumptions of normalcy, and differences among the groups were compared by *t*-test, ANOVA, Chi-square, or Mann-Whitney-*U* test as appropriate.

The factors that explain asthma severity were identified by logistic regression. Our cohort was dichotomized into mild and moderate-severe asthma. We examined the following variables: age, gender, age of onset, skin test positivity, total IgE level, peripheral blood eosinophil count, exposure to tobacco smoke, animal ownership, family history of atopic diseases, LTC₄ synthase genotype, and ALOX5 genotype. Univariate analyses were followed by multivariate logistic regression. A two-sided *P*-value of <0.05 was considered significant.

Results

ALOX5 expression and LTC₄ production by eosinophils as a function of genotype

The age, gender, baseline lung function, FEV₁ response to inhalation of a short-acting beta-agonist, and responsiveness to methacholine were not significantly different in the 5/5 wild-type group and the group bearing only variant alleles (Table 1). In our initial study, there were more individuals of African descent in the deletional variant group; the groups in the replication study were matched for race.

In the initial study, eosinophils from individuals bearing only ALOX5 variant alleles expressed less ALOX5 mRNA than wild-type 5/5 homozygotes (Fig. 1, top). The concentration of LTC₄ in eosinophil supernatants from individuals bearing only variant alleles was significantly lower than that of eosinophils from 5/5 wild-type individuals ($6 \pm 1 \text{ pg/ml}$, $n = 4$ and $21 \pm 5 \text{ pg/ml}$, $n = 5$; $P < 0.05$; Fig. 1, bottom). LTC₄ secretion in this model depends on the ALOX5 product (5-lipoxygenase), because inhibiting its function with 10 μM zileuton decreased leukotriene detection to below 1 pg/ml for both groups ($P < 0.01$ compared with the groups of cells not treated with zileuton).

The results of our confirmatory study were similar to those of our initial study. Eosinophils from individuals bearing only variant alleles expressed less ALOX5 mRNA than eosinophils from homozygous wild-type 5/5 individuals (Fig. 2, top). Again, the concentrations of LTC₄ were significantly lower in the supernatant of cells from individuals bearing only variant alleles than in the supernatant from cells of homozygous wild-type 5/5 individuals ($6.4 \pm 2 \text{ pg/ml}$ and $20 \pm 5 \text{ pg/ml}$, $n = 5$; $P < 0.05$; Fig. 2, bottom).

Eosinophil supernatant LTC₄ levels were not significantly different after stimulation with calcium ionophore (1 μM A23187; $21 \pm 7 \text{ ng/ml}$ 5/5 wild-type; $21 \pm 8 \text{ ng/ml}$ variant-only alleles; $n = 5$).

Studies of asthma severity

A total of 624 children with asthma were entered into the database between October 2002 and September 2003.

Table 1. Characteristics of subjects providing eosinophils

	Age (years)*	Gender (F/M)	Race African-American/ white	FEV ₁ (% predicted)*	β -agonist (%) reversibility*	Methacholine PC ₂₀ (mg/ml)*
Initial study						
Deletional variant	36 $\pm$ 8	3/2	4/1	72 $\pm$ 13	17 $\pm$ 8	1.1 $\pm$ 1
Wild-type	39 $\pm$ 6	3/2	0/5	83 $\pm$ 8	17 $\pm$ 6	0.7 $\pm$ 0.1
Replication study						
Deletional variant	33 $\pm$ 7	1/4	3/2	77 $\pm$ 9	14 $\pm$ 5	2.2 $\pm$ 1
Wild-type	46 $\pm$ 4	2/3	3/2	85 $\pm$ 7	17 $\pm$ 7	1.3 $\pm$ 0.8

*Mean $\pm$ SEM; *P*-values not significant for all wild type – deletional variant comparisons.

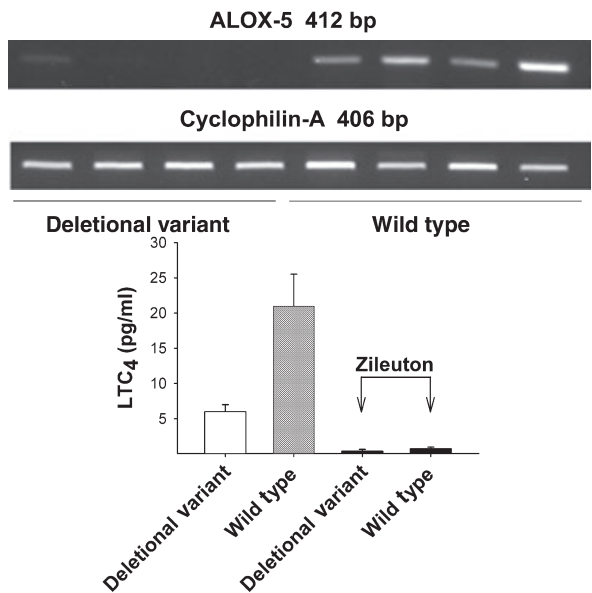


Figure 1. Initial study: ALOX5 mRNA expression ($n = 4$, top) and LTC₄ secretion ($n = 5$, $P < 0.05$ bottom) of eosinophils was lower in cells from individuals bearing two deletional variant than wild-type ALOX5 promoter alleles. LTC₄ secretion is significantly reduced by the 5-lipoxygenase inhibitor zileuton ($P < 0.01$).

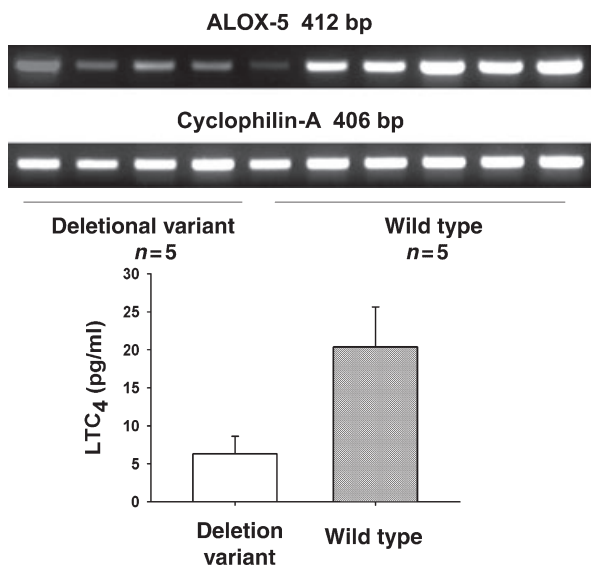


Figure 2. Replication study: ALOX5 mRNA expression (top) and LTC₄ secretion (bottom) of eosinophils from individuals bearing two deletional variant or wild-type copies of the ALOX5 promoter genetic variants ($n = 5$).

ALOX5 genotype could not be discerned in three children, and final analysis was done in 621 children. The demographic characteristics of our cohort of children with asthma are presented in Table 2. The distribution of allele frequencies (Table 3) met the conditions of the

Table 2. Characteristics of the asthmatic children in the asthma severity study

Variable	<i>n</i>	Median	Interquartile range
Age (years)	621	10.6	8.1–13.5
Gender			
Female	222 (35.7%)		
Male	399 (64.3%)		
IgE (kU/l)	616	185	69–489
Eosinophil	611	295	170–490
Skin test			
Positive	395 (64.4%)		
Negative	218 (35.6%)		
FEV ₁ % predicted	611	96	87–105
ALOX5 genotype*			
5/5†	448 (72.1%)		
5/non5	160 (25.8%)		
non5/non5	13 (2.1%)		
LTC4 synthase genotype‡			
AA	372 (60.4%)		
AC	209 (33.9%)		
CC	35 (5.7%)		

*Allele designation refers to the number of tandem Sp1/Egr1 repeats at the ALOX5 core promoter locus.

†LTC4 synthase genotype could not be confirmed in five patients.

Table 3. Characteristics of children with mild and moderate-severe asthma

	Mild (<i>n</i> = 508)	Moderate + severe (<i>n</i> = 113)	<i>P</i> -value
ALOX 5 genotype			
5/5	0.726	0.699	
5/non5	0.260	0.248	0.031*
non5/non5	0.014	0.053	0.008*†
LTC4 synthase			
AA	0.614	0.559	
AC	0.333	0.369	
CC	0.053	0.072	NS*
FEV ₁ %	98 (90–106)	79.0 (72.0–93.0)	<0.001‡
Eosinophil	280 (170–440)	390 (197–600)	0.002‡
IgE	179 (73–451)	229 (51–740)	NS‡

*Chi-square test.

†The more frequent 5 allele is taken as dominant.

‡Mann–Whitney *U*-test.

Hardy–Weinberg equilibrium. Among the 13 subjects bearing only ALOX5 promoter variant alleles, nine had the 4/4, three had the 4/6, and one had the 6/6 genotype.

Our cohort contained 10 subjects with severe asthma. Subjects with moderate-severe asthma had significantly lower FEV₁ and higher peripheral blood eosinophil counts than those with mild disease (Table 3). The non5/non5 ALOX5 genotype was significantly more frequent in the moderate-severe group than in the mild group (5.3% vs. 1.4% $P = 0.008$ by chi-square test).

Univariate analysis identified family history, total IgE, eosinophil count, and ALOX5 genotype as significantly associated with disease severity (Table 4). Multivariate analysis identified family history, eosinophil count, and ALOX5 genotype as predictive of disease severity. The individuals bearing the non5/non5 genotype were more

Table 4. Logistic regression for asthma severity

	Univariate			Multivariate		
	OR	95% CI	P-value	OR	95% CI	P-value
Age	1.033	0.971–1.100	NS			
Female gender	1.129	0.741–1.720	NS			
Age of onset	1.050	0.985–1.121	NS			
Skin test positivity	1.476	0.943–2.311	0.089			
IgE	1.001	1.000–1.001	0.004			
Eosinophil count	1.002	1.001–1.002	<0.001	1.001	1.001–1.002	<0.001
Smoke exposure	0.977	0.707–1.351	NS			
Animal ownership	1.354	0.599–3.065	NS			
Family history	1.567	1.037–2.366	0.03	1.570	1.024–2.405	0.038
LTC ₄ synthase genotype	1.219	0.875–1.697	NS			
ALOX5 genotype*	4.013	1.322–12.181	0.014	3.647	1.146–11.608	0.030

*Common allele (5 Sp1 and Egr1 binding motif repeats) is the dominant.

than three times more likely than those in the combined 5/5 and 5/non5 group to have moderate-severe asthma (OR = 3.647, 95% CI: 1.146–11.608, $P = 0.03$).

Exercise challenge

There were no significant differences in age and gender distribution between the two groups. Children with the non5/non5 genotype ($n = 9$) had more severe exercise-induced bronchoconstriction than those bearing the 5/5 genotype ($n = 40$). The median maximum decline in FEV₁ after exercise was 5% (interquartile range, 2.0–8.0) in the 5/5 group and 13% (6.5–21.0) in the non5/non5 group ($P = 0.02$, Mann–Whitney U -test). Similarly, the AUC after exercise challenge was 36.5 (6.3–81.0) for the 5/5 group and 80.0 (70.0–354.0) for the non5/non5 group ($P = 0.03$, Mann–Whitney U -test).

Discussion

Children bearing only ALOX5 promoter variant alleles are three times more likely to be classified as having moderate or severe asthma than their wild-type-bearing counterparts. In addition to this association with asthma severity as judged by well-established criteria, we also determined the effects of ALOX5 genotype on exercise responses, because they are an objective measure of airway tone. Children bearing only ALOX5 variant alleles had significantly greater bronchomotor responses to exercise testing than did their wild-type-bearing counterparts. Association with asthma severity appears to be specific for the ALOX5 promoter variants, as significant associations were not detected for the LTC₄ synthase promoter variants. These findings constitute two independent lines of evidence that asthma severity in children is a function of ALOX5 promoter genotype.

To better understand how these genetic variants affect the production of LTC₄ in cells that produce cysteinyl-

leukotrienes, we studied eosinophils purified from the peripheral blood of individuals bearing wild-type 5/5 or variant non5/non5 alleles on both chromosomes. Similar to the functional effects of deletional variants derived from cells transfected with reporter constructs (16, 18), RT-PCR studies showed that the eosinophils of asthmatics bearing these variants have impaired ALOX5 transcription. To determine the effects of these differences in ALOX5 transcription on cysteinyl-leukotriene secretion, we measured LTC₄ levels in the eosinophil supernatants. We found congruent changes in baseline LTC₄ secretion, but maximal secretion with Ca-ionophore was unaffected by ALOX5 promoter genotype. Our results, while confirming our previous experience with Ca-ionophore stimulation, extends it by demonstrating that differences in baseline LT production undetectable after 10 min of culture become detectable at 24 hours (32).

Studies of 5-lipoxygenase inhibitors in asthmatics demonstrated that these agents, which inhibit enzyme activity by 30–90%, have clinically relevant effects (33). We observed ALOX-5 promoter genotype-associated reductions in the ability of eosinophils to secrete leukotrienes of this magnitude. Moreover, the proportional reduction in the baseline LTC₄ secretion of eosinophils from individuals bearing only ALOX5 variant alleles compared with that of eosinophils from wild-type 5/5 individuals was similar in magnitude to that observed in peripheral blood cells after oral administration of clinically effective doses of the 5-lipoxygenase inhibitor zileuton (34). The magnitude of the differences in baseline leukotriene secretion we observed may, therefore, be sufficient to produce clinically detectable effects.

The limitation of baseline leukotriene production attributable to ALOX5 variants is compatible with the observation that individuals bearing only deletional variants do not improve their baseline lung function when treated with doses of a 5-lipoxygenase inhibitor that improves baseline lung function in their wild-type 5/5-bearing counterparts (15). This finding suggests that, unlike their wild-type-bearing counterparts, baseline lung function in individuals bearing only deletional variant alleles does not depend on the production of cysteinyl-leukotrienes. Since leukotriene secretion after maximal stimulation is unaffected by these variants, they would not be protective against cysteinyl-leukotrienes released by asthma triggers, including exercise.

We found that children bearing these variants have more severe disease and a greater response to exercise. These findings are mechanistically consistent with a recent study (35) linking ALOX5 deletional variants with increased bronchial responsiveness in patients with acetylsalicylic acid intolerant asthma. While the biologic basis of these observations requires further study, our functional studies provide some useful insights. Leukotriene-producing cells from individuals bearing ALOX5 deletional variants secrete lower concentrations of cysteinyl-leukotrienes at baseline but normal levels after stimulation. Increased

leukotriene sensitivity, resulting from this lower constitutive expression of cysteinyl-leukotrienes, would explain why individuals bearing these variants have more exercise-induced bronchospasm, since their airways would be more sensitive to cysteinyl-leukotrienes released by exercise. Similarly other triggers that release cysteinyl-leukotrienes would result in greater airway narrowing, more dramatic symptoms, and greater requirement for medication.

Alternatively, this association could be because of another genetic variant that is in linkage disequilibrium with the ALOX5 variant, as the functional and case-control studies have been performed in ethnically different populations. However, linkage disequilibrium seems an unlikely explanation, because the undetected variant would have to be common and have direct effects on ALOX5 gene expression larger than any known variant other than the insertion-deletion variant studied (18). While identifying the responsible mechanism(s) will require further study, this association extends our understanding of exercise-induced bronchospasm, because it identifies genetic differences as one determinant of this understudied aspect of the asthma phenotype.

Our study has limitations. While the non5/non5 genotype is an important determinant of asthma severity, it accounts for approximately 2.1% of Ankara, 4.4% of European-American, and 9.0% of African-American asthmatics. Despite studying a cohort of substantial size, we could only include a limited number of children bearing two variant alleles, which increased the likelihood that our findings could be because of type I error. To address this issue, we phenotyped our children using a second and objective measure of

asthma severity. Finding consistent results substantially reduces the likelihood that these associations occurred by play of chance. Similarly, the low frequency of asthmatics bearing only deletional variants prevented us from obtaining eosinophils from a large numbers of subjects. We could perform and replicate a study that was adequately powered to detect differences in leukotriene secretion of a magnitude that had been shown to be clinically significant in pharmacological studies (33, 34). Additionally, our findings are consistent with both *in vitro* (16) and pharmacogenetic studies (15). However, clearly, because of the low prevalence of asthmatic children with variant forms of this gene, the clinical relevance of our findings is low.

Taken together, our findings suggest that children who express the asthma phenotype despite having a genetic variant that impairs their ability to express ALOX5 have more severe disease than those bearing genotypes that have more efficient baseline expression of ALOX5.

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