

Genetics and the variability of treatment response in asthma

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Pharmacogenetics is the study of how genetic differences influence the variability in patients' responses to therapy. Using specific examples in asthma, I will define the concepts of variability in response to therapy in asthma. I will then use the relationship between specific genetic polymorphisms and the response to β -adrenergic agonists and leukotriene modifiers to illustrate pharmacogenetic interactions and to acquaint the reader with the applicability of these findings to clinical practice. (*J Allergy Clin Immunol* 2005;115:S532-8.)

Key words: *Asthma, treatment response variability, pharmacogenomics, leukotrienes, beta-agonists*

The prior article has given us an overview of genomic discoveries that suggest that multiple genetic variables can contribute to asthma and asthma susceptibility. In the material that follows, we will discuss evidence that suggests that among individuals with asthma, genomic advances will permit us to identify polymorphisms associated with individual variability in response to therapy. The field of such study, known as pharmacogenetics, is the study of how genetic differences influence the variability in patients' responses, both therapeutic and adverse, to therapy.

This discussion of pharmacogenetics and asthma is not meant to be a comprehensive overview of the field. Rather, this article will highlight selected areas to illustrate the concepts and advances in the field. After a clinical case example relevant to asthma, I will discuss the concept of variability in response to asthma therapies, using inhaled corticosteroids as the paradigm. Then I will briefly review the principles of pharmacogenetics. Lastly, I will address genetic polymorphisms in 2 candidate pathways that have been associated with alterations in therapeutic responses: (1) the effect of genetic polymorphisms of the β -adrenergic receptor and (2) polymorphisms of 5-lipoxygenase pathway enzymes.

CASE REPORT

The following case illustrates how "one person's cure can be another person's poison." A 27-year-old male with

Abbreviation used

ACRN: Asthma Clinical Research Network

severe asthma since childhood was referred for evaluation. The patient had multiple hospitalizations since childhood and was unable to reduce oral prednisone to less than 15 mg/d for the past 2 years. He complained that inhaled corticosteroids were ineffective and increased his wheezing. He was using a nebulizer to administer β -agonist therapy 10 to 12 times each day and an additional 4 times at night. Fear of exacerbating his asthma prevented him from working and leaving the house. On examination, he was morbidly obese and had scattered wheeze. His FEV₁ was 70% of predicted value, and his sinus computed tomographic scan showed mucosal thickening. Mild reflux was observed in his pH probe, and his laboratory evaluation was remarkable for only 7% eosinophils. There was concern that high-dose β -agonist therapy was aggravating rather than ameliorating his disease. High-dose inhaled ipratropium bromide and montelukast were prescribed. Additionally, a schedule was established to taper his β -adrenergic agonist use. Over the course of 6 weeks, he was able to taper his use dramatically. Three months later, he was using β -agonists occasionally during the week, and he was no longer requiring oral prednisone.

VARIABILITY IN RESPONSES TO THERAPY: INHALED CORTICOSTEROIDS AS A MODEL

Pharmacogenetics relates genetic variability to variability in responses to therapy. Although we are aware that there is tremendous variability in the genetic code among individuals (single nucleotide polymorphisms [alteration in a single base pair] occur on average once in every thousand base pairs), we are not accustomed to conceiving of the variability that occurs in responses to the therapies we use. We will use the response to inhaled corticosteroids to illustrate variability in beneficial and adverse effects.

Although we previously had thought that responses to corticosteroids are uniform, a study by Malmstrom et al¹ showed that they are not. In this study treatment with an inhaled corticosteroid (beclomethasone) was compared with treatment with a leukotriene antagonist (montelukast) and placebo. Percentage change in FEV₁ was the measured outcome. Asthmatic subjects treated with beclomethasone exhibited a mean 12% improvement in FEV₁, whereas patients treated with montelukast exhibited a

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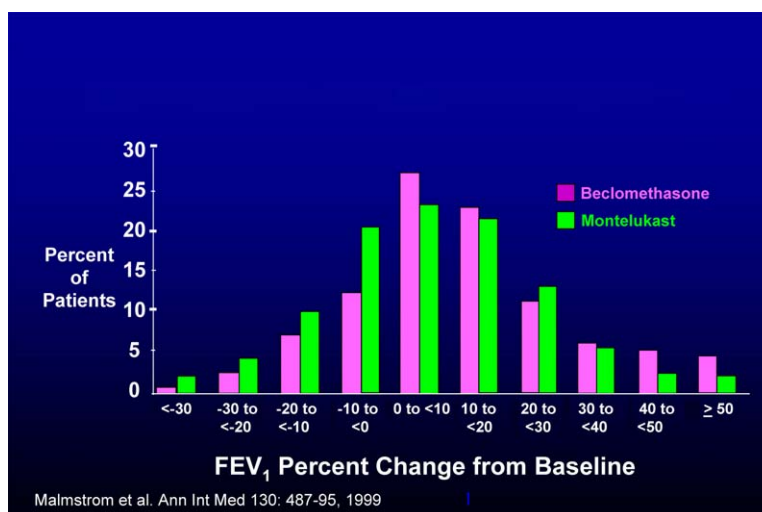


FIG 1. Distribution of patients experiencing a particular change in FEV₁. Green bars represent patients receiving montelukast, 10 mg once daily; pink bars represent patients receiving inhaled beclomethasone, 200 µg twice daily. Adapted with permission from Malmstrom et al. *Annals of Internal Medicine* 1999.¹

mean 7% improvement.¹ An examination of the distribution of the individual responses revealed that the average response for beclomethasone was driven to a large extent by subjects who are hyperresponders (ie, patients with >40% improvement in their FEV₁). Furthermore, up to 25% of patients had minimal response to beclomethasone (Fig 1).¹ The fact that not all patients respond to inhaled corticosteroids is reinforced by a study from the National Institutes of Health Asthma Clinical Research Network (ACRN). That study showed that even after treatment with up to 1200 µg of inhaled beclomethasone followed by a period of using 2000 µg of inhaled fluticasone, more than 30% of patients did not have a response, as judged on the basis of FEV₁.² A similar degree of nonresponsiveness was observed when fluticasone was used as the inhaled corticosteroid.

In addition to variability in therapeutic responses, there exists a variability of response in regard to adverse effects. In a study that investigated the relationship between increasing doses of inhaled corticosteroids and a decrease in bone density in premenopausal women,³ a significant relationship was observed between increasing use of triamcinolone per day and the decrease in bone density. An examination of the individual responses showed that some patients were losing bone at a rapid rate, whereas other patients were not losing bone at all (Fig 2).³

PHARMACOGENETIC MECHANISMS

It is estimated that up to 70% of the variability in therapeutic responses to pharmacotherapy might be due to genetic variability.⁴ By what mechanisms can genetic polymorphisms produce such effects? Genetic alterations can affect 3 areas that can alter responses: (1) variations that affect drug metabolism, (2) variations that alter unintended targets that can lead to adverse effects, or (3)

variations in the therapeutic target itself or pathways involved in producing its effects.

What types of drug responses are candidates for pharmacogenetic exploration? From a clinical point of view, there are 2 key characteristics. First, as outlined previously, there needs to be variability in response. Second, individual response should be repeatable.

When such a response has been identified, how does one identify genetic variability that might account for that response? On the basis of an understanding of the mechanism of action of the intervention, one can search for genetic variants in biologic pathways involved in the drug's therapeutic and toxic effects. Traditionally, this has involved identifying polymorphisms that produce biologically plausible effects and examining whether those effects alter clinical responses. The results of this approach will be reviewed below. A nonheuristic approach to identification of polymorphisms in which statistical analysis of relationships between responses and polymorphisms of unknown biologic significance is performed can also be fruitful, but examples will not be emphasized in this brief review.

β-ADRENERGIC RECEPTOR POLYMORPHISMS

β-Agonists act at the β-adrenergic receptor to produce bronchial smooth muscle relaxation. Reihsaus et al⁵ described several polymorphisms in the gene coding for the β-adrenergic receptor. Several of these polymorphisms produce amino acid changes. Some of these variants are rare. However, changes at the 16th and 27th amino acid positions of the receptor occur frequently enough to postulate that they might account for some of the clinically observed variability in response to β-agonists. Variants at positions 16 and 27 do not affect

Yearly Δ in Bone Density: Total Hip

Israel, et al., *NEJM* 2001

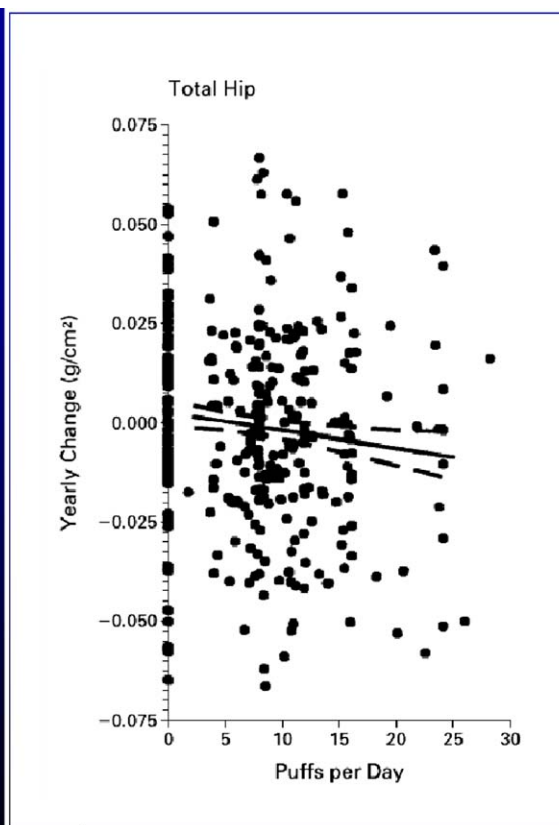


FIG 2. Distribution of yearly change in hip bone density in relation to the average number of daily puffs of inhaled corticosteroid. The *solid line* represents the mean change. The *dashed lines* indicate the 95 percent confidence interval for the mean. Adapted with permission from Israel EBT, Fitzmaurice GM, Kotlov TV, LaHive K, LeBoff MS. *N Engl J Med.* 2001;345:941-7. Copyright © 2001 Massachusetts Medical Society. All rights reserved.³

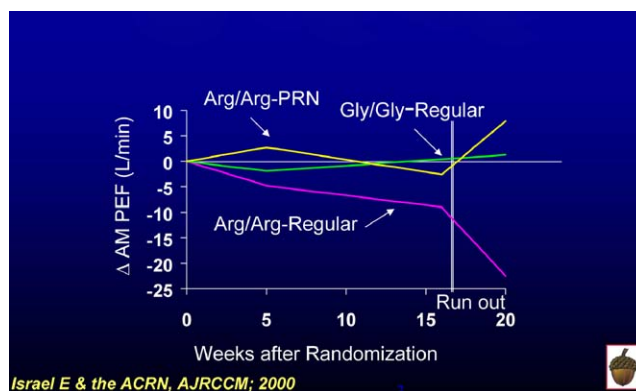


FIG 3. Effect of genetic polymorphisms at position 16 of the β_2 -agonist on AM PEF in patients using regular albuterol versus prn for 16 weeks followed by a 4 week run-out. Arg/Arg patients who received regularly scheduled beta-agonist treatment (Arg/Arg-Regular) experienced a 30.5 ± 12.1 L/min decline in AM peak expiratory flow relative to those who received as-needed treatment (Arg/Arg-PRN; $P = 0.012$). Gly/Gly patients were not affected by regular treatment (Gly/Gly-Regular). Adapted with permission from Israel et al. *American Journal of Respiratory and Critical Care Medicine* 2000.⁹

agonist-receptor coupling; however, they do alter receptor downregulation.⁵ Furthermore, it has also been shown that haplotypic combinations of single nucleotide polymorphisms or variants in this gene can affect protein and

transcript expression. Drysdale et al⁶ demonstrated that different haplotype combinations that affect the production of the β -agonist receptor also affect the response to β -agonists.

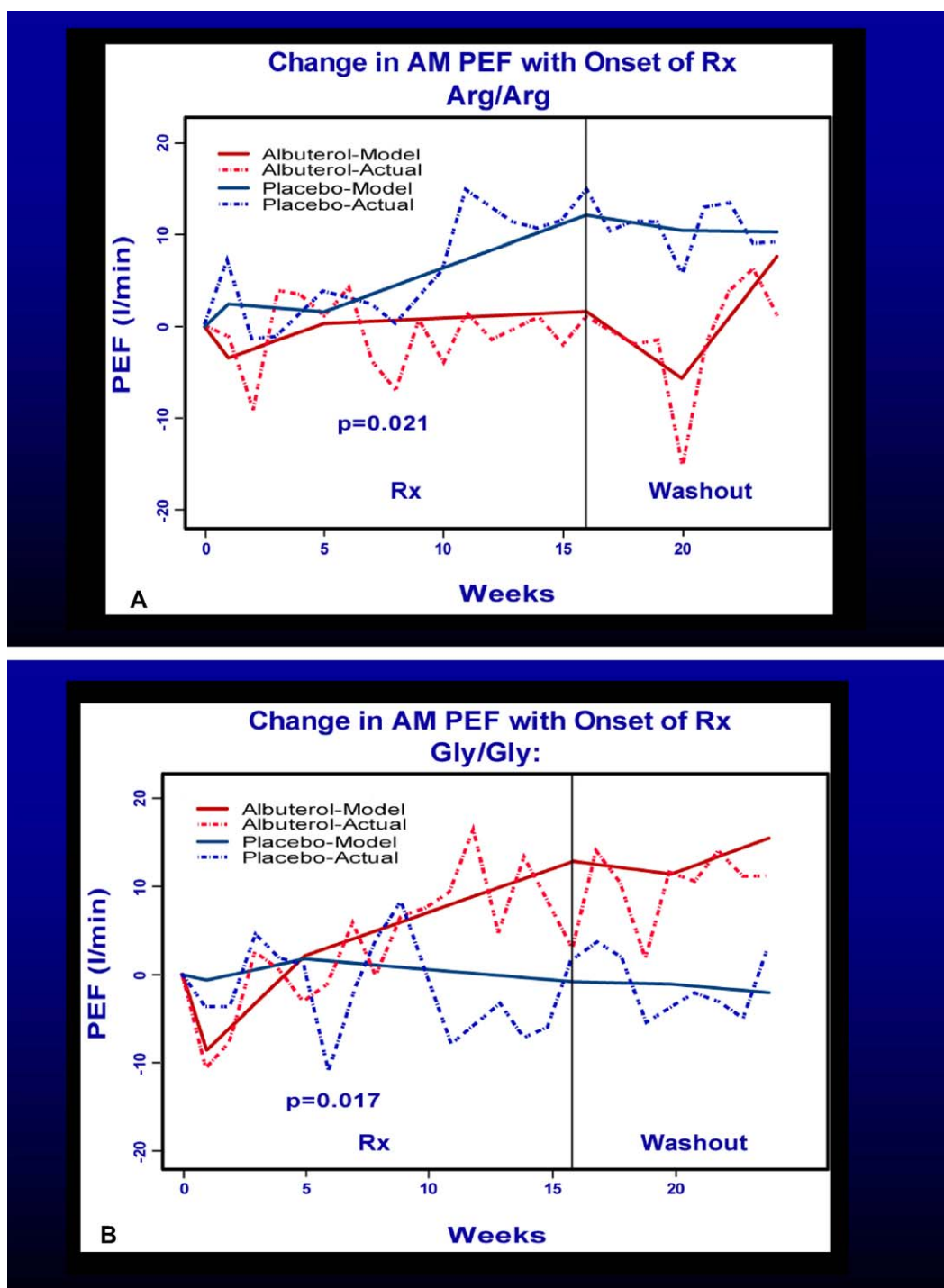


FIG 4. Effect of regular albuterol (Albuterol) versus placebo albuterol (Placebo) on AM PEFR during the 16 week treatment period and 8 week washout within each genotype at the 16th position of the β_2 -agonist. **A**, Arg/Arg. **B**, Gly/Gly. *Wild type*, 5 binding motif repeat alleles; *mutant*, non-5 repeat alleles; *heterozygote*, 1 mutant + 1 wild-type allele. Patients with the Arg/Arg genotype exhibited significantly improved PEFR in the placebo phase more than the albuterol phase (10 L/min; $P = 0.0209$). In contrast, patients with the Gly/Gly genotype experienced greater improvement in their PEFR during the regular albuterol phase as compared to the placebo phase (14 L/min; $P = 0.0175$). Reprinted with permission from Israel E, Chinchilli VM, Ford JG, et al. Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomized, placebo-controlled cross-over trial. *Lancet* 2004;364:1505-12.¹¹

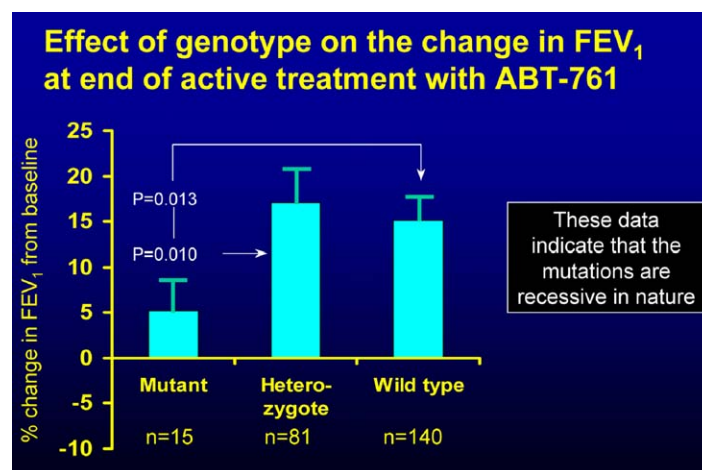


FIG 5. Percent change in FEV₁ from baseline for patients with mutations at the *ALOX5* core promoter locus treated with the 5-lipoxygenase inhibitor ABT-761. Adapted with permission from Drazen et al. Copyright © 1999 *Nature Genetics*.¹⁵

In the early 1990s, there was concern about whether regular use of β -agonists might produce adverse effects in asthmatic subjects. A study had demonstrated that patients treated with β -agonists on an as-needed basis have fewer exacerbations than patients treated with a regular regimen of β -agonists.⁷ This suggested that treatment with a β -agonist might not always be beneficial. As a result, the National Institutes of Health ACRN undertook a study to investigate whether regular β -agonist use produced adverse effects in patients with mild-to-moderate asthma. The ACRN demonstrated that there was no difference in terms of morning peak flow and other outcomes in these patients, irrespective of whether they were treated regularly or intermittently with β -agonists.⁸ However, there was no improvement with regular use, and the group concluded that these patients should not be instructed to use β -agonists regularly because such therapy produced no improvement compared with intermittent use.

After the description of the polymorphisms in the β -adrenergic receptor at positions 16 and 27, the ACRN re-evaluated the patients in the initial study. The variation at position 27 had no effect on any of the responses; however, the variant at position 16 did have an effect on the response to β -agonists (Fig 3).⁹ Patients homozygous for arginine at position 16 (Arg/Arg) treated regularly with β -agonists experienced a decrease in peak flow over the period of the study. In contrast, patients treated regularly with β -agonists who were homozygous for glycine at position 16 (Gly/Gly) did not experience a change in their peak flow throughout the study. Furthermore, Arg/Arg patients receiving intermittent treatment with a β -agonist did not experience a change in their peak flow. The effect was most apparent after the patients had been returned to as-needed β -agonist use. The observation that Arg/Arg might be associated with adverse effects was supported by Taylor et al¹⁰ in a study that included patients treated

regularly with albuterol. The Arg/Arg patients experienced a doubling in exacerbations with regular albuterol treatment, whereas such an increase did not occur in the Gly/Gly patients or those heterozygous at the 16th position.

These retrospective analyses stimulated the ACRN to undertake a prospective, randomized, placebo-controlled, double-blind trial of regular versus minimal albuterol treatment by genotype known as the Beta-Agonist Response by Genotype study.¹¹ Patients were genotyped and assigned to groups on the basis of homozygous genotype at the 16th amino acid position, treated with regular or intermittent therapy, and then crossed over. To minimize β -agonist use, patients were provided ipratropium bromide for rescue use and instructed only to use albuterol if symptoms were not relieved with ipratropium bromide. Arg/Arg patients experienced decreased peak flow when treated with regular β -agonist compared with placebo. In contrast, Gly/Gly patients had a significant improvement in their peak flow when they were treated with regular β -agonists compared with placebo (Fig 4). Similar effects were seen in FEV₁. After 16 weeks of exposure, the combined difference in FEV₁ between Arg/Arg and Gly/Gly patients treated regularly with β -agonists versus those treated with as-needed β -agonists and anticholinergic agents was almost 200 mL. These effects were not limited to indices of airway function. Arg/Arg patients experienced an increase in their daily symptoms while using regular compared with as-needed β -agonists. In contrast, Gly/Gly patients had a significant decrease in their symptoms. Arg/Arg patients even increased their supplemental ipratropium bromide and supplemental albuterol use when they were treated regularly with β -agonists compared with as-needed β -agonist therapy. Furthermore, during the run-in period of this study, in which patients minimized their β -agonist use and used an anticholinergic agent, Arg/Arg patients experienced an

increase in their peak flow compared with that seen in Gly/Gly patients, suggesting that even as-needed β -agonist use might be deleterious in Arg/Arg patients. This study suggests that Arg/Arg asthmatic subjects might experience adverse effects from use of regular β -agonists rather than benefiting from the treatment.

POLYMORPHISMS OF THE 5-LIPOXYGENASE PROMOTER

The leukotriene modifiers act by preventing the production or action of leukotrienes. Although there has been a possible association detected between polymorphisms of the leukotriene C_4 synthase enzyme and responses to leukotriene modifiers,¹² for the purposes of illustration in this review, we will focus on polymorphisms of the 5-lipoxygenase enzyme.

The leukotrienes are synthesized through a pathway that depends on the enzyme 5-lipoxygenase. The 5-lipoxygenase promoter has been sequenced and has been shown to possess a regulatory region with a binding motif for an upregulating binding factor.¹³ Most individuals have 5 repeats of this positive binding motif; however, others might have as many as 6 to 7 or as few as 3 to 4 repeats. Addition or deletion of these copies of the binding motif appears to change the efficiency of 5-lipoxygenase and results in less leukotriene production.¹⁴ Approximately 5% of patients are homozygous for an altered (non-5-repeat) 5-lipoxygenase promoter. It was hypothesized that patients possessing the altered promoter might be less responsive to a leukotriene modifier. This hypothesis was investigated by stratifying the results of a clinical study of a 5-lipoxygenase inhibitor, irrespective of whether patients had none (homozygous wild type), 1 (heterozygous), or 2 (homozygous mutant) copies of the alternative version of the gene. The primary outcome of the clinical study was improvement in FEV₁. In the unstratified population, the inhibitor produced a 12% to 14% improvement in FEV₁. Patients homozygous for the wild-type promoter had a 15% improvement in FEV₁. In contrast, those patients homozygous for the mutant version of the promoter had a significantly decreased FEV₁ response (Fig 5).¹⁵

It is important to note that the pharmacogenetic interactions described are not restricted to therapeutic interactions in asthma alone, nor are the associations with genetic polymorphisms restricted solely to drug therapy. For example, the alterations discussed above in the 5-lipoxygenase promoter have been investigated in relationship to the development of atherosclerosis in association with diets high and low in arachidonic acid, which is a substrate for the 5-lipoxygenase enzyme.¹⁶ An investigation was undertaken on the basis of the hypothesis that inflammation in the blood vessels contributes to the development of atherosclerosis and that alterations in the 5-lipoxygenase promoter would affect the pattern of inflammation and the resultant interaction effect of dietary alterations. Intimal thickness was used as the primary indicator of vessel atheroscle-

rosis. Patients homozygous for the mutant form of the promoter demonstrated a greater risk for increased intimal thickness in association with high arachidonic acid intake than those with the nonmutant forms.¹⁶ These findings provide another example of the effect of genetic variability on the responses to therapeutic (in this case dietary) interventions.

CONCLUSION

There is individual variability in the responses to pharmacotherapeutic and environmental interventions. A significant proportion of the variability relates to individual genetic differences. Technology to rapidly detect particular genetic polymorphisms is rapidly evolving. It is likely that in the near future, information regarding individual genetic polymorphisms will allow us to identify those patients most likely to respond, those likely to experience adverse effects from therapeutic interventions in asthma, or both, as well as most therapeutic areas in medicine.

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