

Polymorphisms at the glutathione S-transferase, GSTP1 locus: a novel mechanism for susceptibility and development of atopic airway inflammation

A common feature of environmental irritants is their ability to cause local inflammation which could alter airway function. The principal targets of such injury are the epithelial cells lining the airway passages and the lower respiratory gas-exchange areas. While host atopy is a recognized risk factor for airway inflammation, atopy alone cannot cause asthma. We hypothesize that susceptibility to persistent airway inflammation in atopic individuals is characterized by an inherited deficiency in the effectiveness of detoxification of inhaled irritants and products of oxidative stress such as reactive oxygen species (ROS). Our case-control studies show that polymorphisms at the glutathione S-transferase, GSTP1, locus on chromosome 11q13 may account for variation in host response to oxidative stress, a key component of airway inflammation. Frequency of the GSTP1 Val/Val genotype is reduced in atopic subjects compared with nonatopic subjects. Trend analysis also shows a significant decrease of GSTP1 Val/Val (with parallel increase of GSTP1 Ile/Ile) genotype frequency with increasing severity of airflow obstruction/bronchial hyperresponsiveness. The implication of specific polymorphisms at the GSTP1 locus in airway inflammation is entirely novel: however, GST are recognized as a supergene family of enzymes critical in 1) cell protection from the toxic products of ROS-mediated reactions, 2) modulation of eicosanoid synthesis.

M. A. Spiteri, A. Bianco

Lung Injury and Inflammation Group, Department of Respiratory Medicine, Keele University, Staffordshire, UK

R. C. Strange, A. A. Fryer

Clinical Biochemistry Research Laboratory, Centre for Cell and Molecular Medicine, Keele University, Staffordshire, UK

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Dr Monica A. Spiteri

Lung Injury and Inflammation Group
Department of Respiratory Medicine
North Staffordshire Hospital
Stoke-on-Trent
Staffordshire ST4 6QG
UK

Tel. +44-1782-552332

Fax: +44-1782-552323

Email: m.spiteri@virgin.net

Introduction

Asthma is on the increase, and epidemiologic studies indicate that the incidence of intermittent wheeze, clinical asthma, eczema, and hay fever has nearly doubled over the past 25 years (1). While present knowledge strongly implicates host genetic factors, it is equally probable that environmental factors also have a crucial interactive role in determining whether susceptible individuals develop such allergic diseases.

Breathing exposes the lung to many irritants that can alter airway function. However, the mechanism/s whereby environmental agents including aeroallergens (e.g., grass pollens) (2) and pollutants (e.g., ozone) (3) cause airway inflammatory diseases such as asthma remains unclear. Furthermore, while exposure to irritants is critical, atopy is a recognized risk factor for airway inflammatory diseases. Yet, although 30–50% of the population is atopic, only 5–7% develop asthma. Thus, while asthma has an allergic component, atopy is not sufficient to cause the disease. In addition, it is unknown how varied inhaled irritants damage airway cells, and how this leads to bronchial hyperresponsiveness (BHR) and airway obstruction in a subgroup of atopic subjects.

Oxidative stress: critical component of airway inflammation

A common feature of environmental irritants is their ability to cause local inflammation. For example, ozone at ambient levels causes respiratory symptoms characterized by airway inflammation and consequent local formation of reactive oxygen species (ROS) (4). While, ideally, the bronchial airway will be protected from such cytotoxic products, it is clear that individuals differ in their ability to deal with an oxidant burden, and that, in part, these differences are genetically determined. We propose that such differences in host detoxification ability provide the basis for either resolution or progression of inflammation in atopic individuals after exposure to an environmental trigger (Fig. 1). Thus, while breathing constantly exposes the lung to numerous irritants that can initiate the development and persistence of airway inflammation, this is clinically reflected only in certain individuals. Furthermore, symptom severity varies among affected subjects such that some patients with allergic airway disease have little impairment, while others have debilitating breathlessness.

The principal targets for lung injury are the epithelial cells (EC) lining the air passages and gas-exchange regions. Although the kinds of reactive intermediates

and initial toxic mechanisms involved may vary with different irritants, the end result is a cellular inflammatory response with the production of ROS. Studies with environmental irritants (e.g., ozone and oxides of nitrogen [5, 6]) show that these gases are consumed by reaction with components of EC and/or lining fluid, leading to increased production of ROS. These in turn react with biomolecules in the airway, giving rise to cytotoxic, oxidized DNA and lipid products including hydroperoxides and aldehydes. In most nonatopic and atopic individuals, airway EC will, via enzyme systems (7) and scavengers (8), metabolize these products. We hypothesize that in high-risk, atopic individuals, the EC are unable to metabolize ROS products efficiently, leading to uncompensated oxidant stress in the lung. This triggers a chain of events, including release of chemotactic factors for the activation and recruitment of immune effector cells (which precipitates further release of ROS), and unveiling of bronchoconstrictor mechanisms. The latter result from an upregulation of bronchoconstrictor eicosanoids ($\text{PGF}_{2\alpha}$, LTB_4 , $\text{LTC}_4\text{-E}_4$) and simultaneous inhibition of bronchoprotective eicosanoid production (PGE_2 , PGI_2) through selective expression of key rate-limiting enzymes in the arachidonic acid pathway, either by the direct effect of lipid hydroperoxides and hydroxyl radicals on eicosanoid synthesis (9, 10), or via release from EC after insult (11). Clinically, this is reflected in the onset or aggravation of respiratory symptoms.

Genetic variation in ROS detoxification: GST polymorphisms

Inherited susceptibility (to persistent inflammation) and/or outcome (assessed by disease symptoms and severity) depends on genetic factors that mediate various aspects of the disorder. For example, identification of the genes that influence response to inhaled agents, such as the specific IgE response and the IL-4 gene cluster, has attracted much interest (12). However, it is recognized that genetic susceptibility to allergic asthma is likely to result from simultaneous inheritance of several mutant or polymorphic genes. Selection of appropriate candidate susceptibility/outcome genes for any disease may be problematic. Our strategy has been to use a polymorphic candidate gene (rather than microsatellite) approach based on specific genes with an established role in cellular protection against oxidative stress, thereby critically influencing prostaglandin (PG) and leukotriene (LT) production (which serve as key mediators of the atopic asthmatic response). In this respect, members of the glutathione S-transferase (GST) supergene family are attractive, as an accumulating body of evidence indicates that the enzymes of the mu (GSTM1, GSTM3), theta (GSTT1), and pi (GSTP1) classes are critical in the protection of cells from the toxic products of ROS-mediated reactions (lipid and DNA hydroperoxides, aldehydes) (13). Furthermore, many cytosolic GST (particularly mu and pi class enzymes) demonstrate activity toward both leukotrienes and prostaglandins

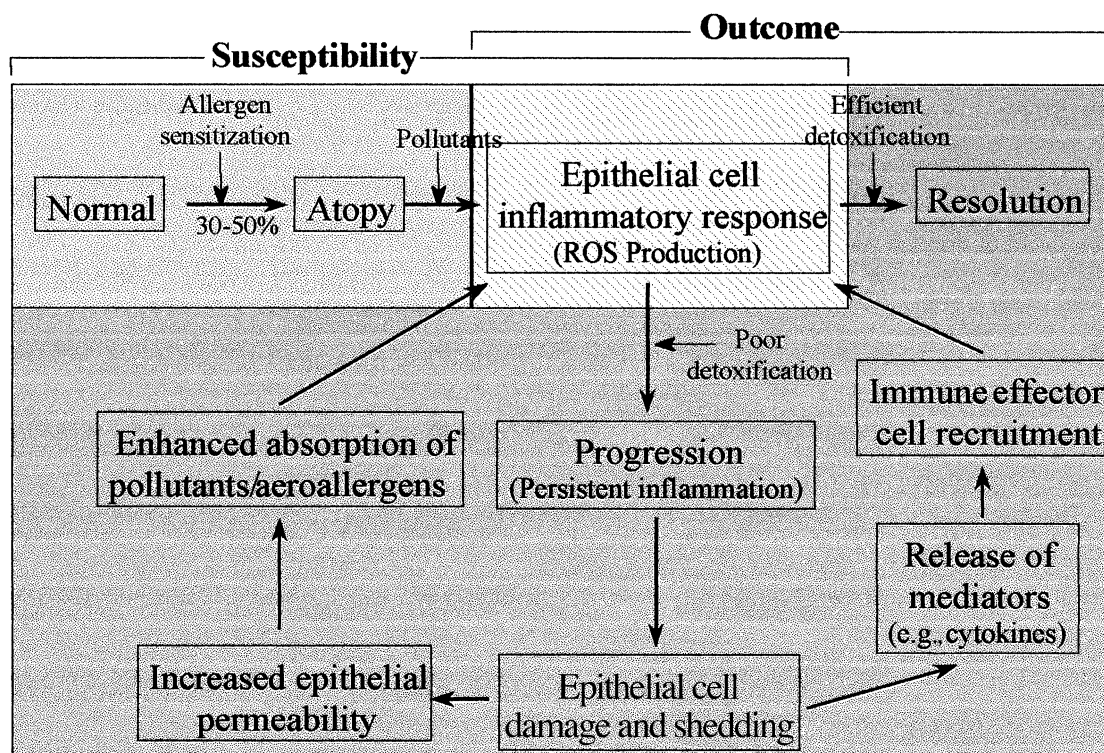


Figure 1. Schematic diagram of putative effect of detoxification of reactive oxygen species (ROS) after inflammatory insult within airway.

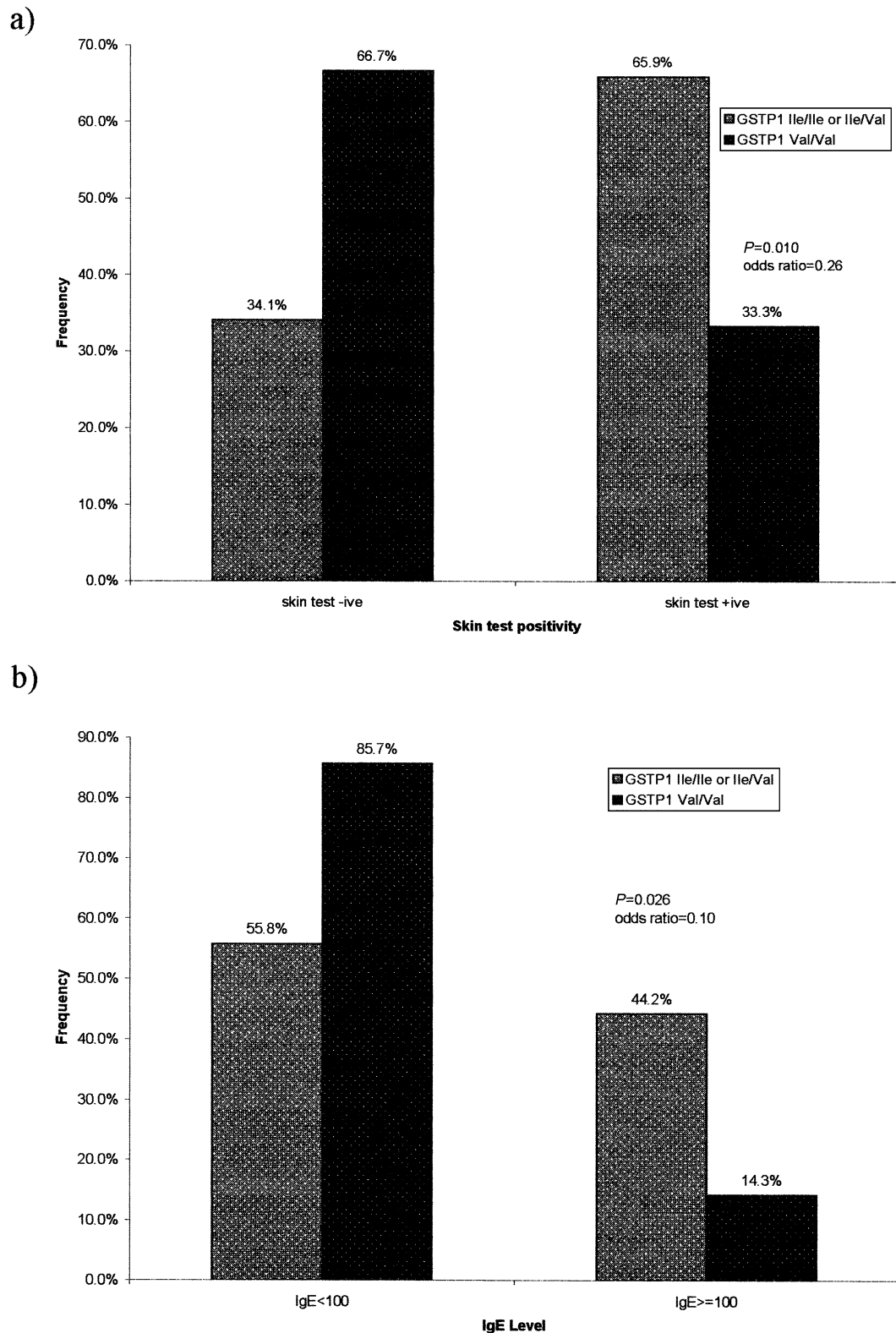


Figure 2. Association of GSTP1 genotype with atopic indices: a) skin test positivity; b) IgE level.

and may therefore influence eicosanoid synthesis either directly or via the modulation of the level of lipid hydroperoxides (known to induce eicosanoid synthesis, together with other ROS).

Each of these GST genes is polymorphic with, for example, homozygosity for the deleted *GSTM1*0* and *GSTT1*0* alleles linked with risk of several inflammatory diseases (14). Our interest is focused on GSTP1,

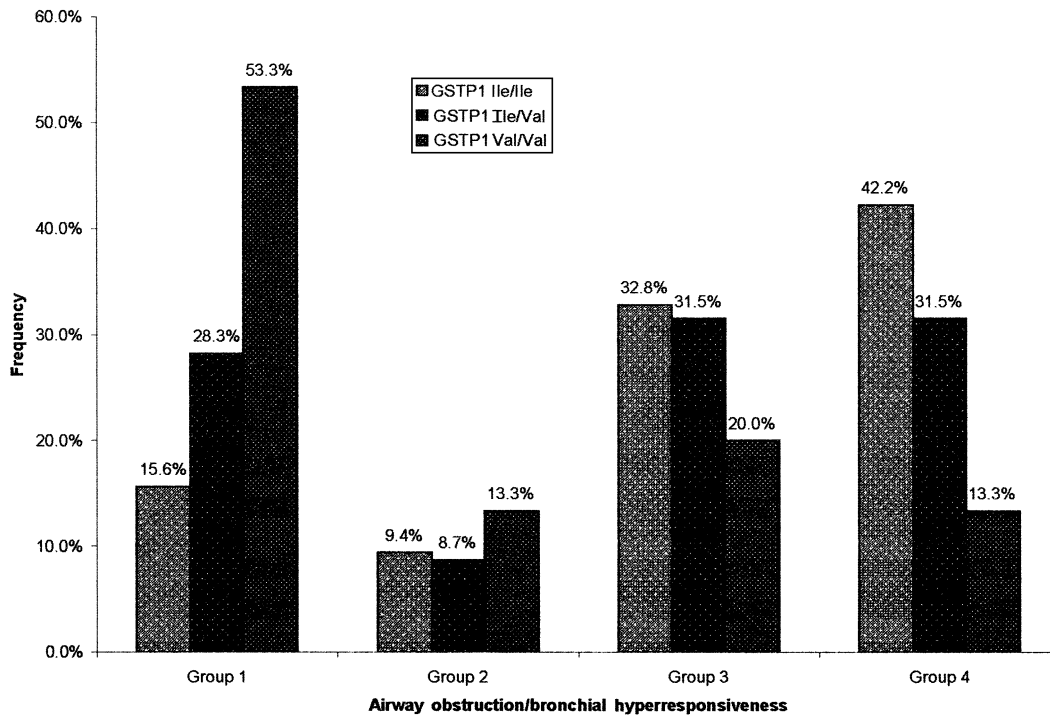


Figure 3. Association of GSTP1 genotype with airway obstruction/bronchial hyperresponsiveness. Subjects were stratified into four groups: group 1) FEV₁ > 80% predicted and PC₂₀ > 16 mg/ml (BHR negative); group 2) FEV₁ > 80% predicted and PC₂₀ positive with dose of methacholine of 8–16 mg/ml (borderline BHR); group 3) FEV₁ > 80% predicted and PC₂₀ positive with dose of methacholine of 0.03–8 mg/ml (BHR positive); group 4) FEV₁ ≤ 80% predicted (severe airway dysfunction). Trend test analysis showed that proportion of subjects with GSTP1 Ile/Ile increased in parallel with degree of airway reactivity/obstruction, while proportion of Val homozygotes demonstrated inverse trend (GSTP1 Ile/Ile; reference genotype, GSTP1 Ile/Val; $P=0.072$, GSTP1 Val/Val; $P=0.002$).

which has also been shown to be polymorphic with allelic variants that are both common and functionally relevant (15). Thus, in addition to the wild-type, *GSTP1**A allele, three more alleles have been identified: *GSTP1**B, *GSTP1**C, and *GSTP1**D (16–19). *GSTP1**B contains an A–G transition at nucleotide +313, resulting in an Ile¹⁰⁵-Val¹⁰⁵ (I105V) amino-acid change, while *GSTP1**D contains a C–T transition giving rise to an Ala¹¹⁴-Val¹¹⁴ (A114V) substitution. *GSTP1**C contains both these transitions. Enzymes with the valine at amino-acid 105 have a sevenfold higher catalytic efficiency for the diol epoxides of polycyclic aromatic hydrocarbons (PAH) than the isoenzymes with the isoleucine at this position. In contrast, the Val¹⁰⁵ enzyme is threefold less effective with 1-chloro-2,4-dinitrobenzene as substrate (15, 17, 18). There is still no evidence of a functional effect of the A114V substitution alone (*GSTP1**D), although it has been suggested that it augments the increased PAH activity of the I105V substitution (*GSTP1**C) (15). The differences in catalytic efficiencies of the polymorphic variants appear to be due to induced changes in active site architecture (15). In our initial studies (summarized in Figs. 2 and 3) (20), we focused on the +313 polymorphism (I105V) and designated homozygotes for this transition, GSTP1 Val/Val. These subjects therefore include *GSTP1**B homozygotes, *GSTP1**C

homozygotes, and *GSTP1**B/*GSTP1**C compound heterozygotes. Similarly, patients with GSTP1 Ile/Ile include *GSTP1**A homozygotes, *GSTP1**D homozygotes, and *GSTP1**A/*GSTP1**D compound heterozygotes. The GSTP1 gene, located on chromosome 11q13, encodes the predominant cytosolic GST enzyme expressed in human lung (21, 22). We have confirmed this in both adult and foetal human lung by both activity (23) and mass measurements (24). Using in-house antibodies specific to lung GSTP1, we have further shown that GSTP1 expression is localized to the epithelial cells lining the major airways, with some alveolar epithelial cells demonstrating positivity (25, 26). Members of the mu (GSTM1, GSTM3), alpha (GSTA1/2), and theta (GSTT1) families are also expressed in lung epithelium, although to a lesser degree.

The importance of GSTP1 polymorphisms in atopic airway disease is indicated by our case-control data (20) showing that the frequency of the GSTP1 Val/Val genotype was reduced in atopic subjects compared with nonatopic controls (Fig. 2). The GSTP1 genotype was also significantly associated with the severity of airway dysfunction as defined by BHR. Thus, trend test analysis demonstrated a significant decrease of GSTP1 Val/Val (and parallel increase of GSTP1 Ile/Ile) genotype frequency with increasing airway dysfunc-

tion (Fig. 3). We have also examined associations between the GSTP1 A114V substitution and atopy/BHR. We have shown that unequivocal identification of the *GSTP1**A, *GSTP1**B, *GSTP1**C, and *GSTP1**D alleles does not add to the effect noted by studying the I105V substitution. In addition, differences in the frequency of the GSTM1, GSTM3, and GSTT1 genotypes did not achieve statistical significance.

The association between the GSTP1 genotype and severity of airway dysfunction remained significant after correction for potential confounding factors (age, sex, skin prick tests, and IgE levels). These results suggest that putatively high-activity GSTP1 genotypes have potential implications for individual susceptibility to the damaging effects of ROS, and that altered activity, determined by detoxifying enzyme phenotype (or variation in inducibility of these genes) at the level of the EC, may influence the inflammatory response in atopic subjects. While development of asthma in the atopic individual is determined by complex interactions between genetic and environmental factors, separate studies have shown that individuals with lowered antioxidant capacity are at increased risk of allergic asthma (27). Furthermore, BHR is a consistent key clinical feature of asthma, although it demonstrates inter- and intraindividual variability. The mechanism of BHR is unclear, although it probably involves components of increased mucosal permeability, modulation of reflex pathways, and sensitization of airway smooth muscle. Our finding that BHR is closely linked to specific GSTP1 polymorphisms supports the view that this GST plays a critical part in facilitating development of airway hyperresponsiveness.

Functional significance of GST polymorphisms in airway inflammation

Advances in asthma management are likely to depend on a better understanding of how genetic and environmental factors influence susceptibility to, and outcome in, this disease. The implication of specific polymorphism at the GSTP1 locus in the pathogenesis of allergic asthma is entirely novel. Our studies have shown that the presence of the A nucleotide at position +313 (Ile¹⁰⁵) is associated with increased risk of BHR and atopy, while the valine-encoding variant (Val¹⁰⁵) allele confers reduced risk (20). We propose that the GSTP1 polymorphism, located on chromosome 11q13, provides the crucial link between

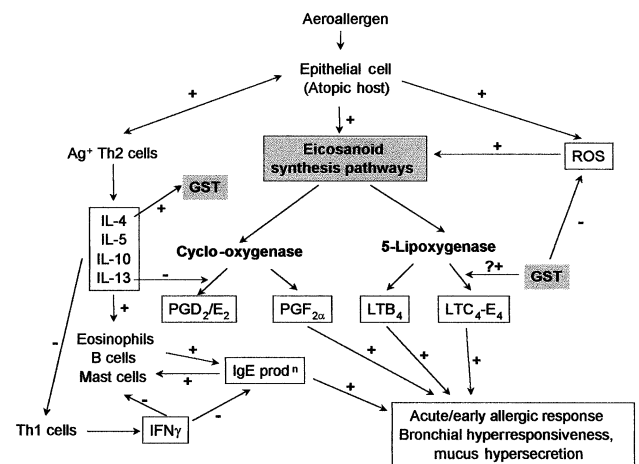


Figure 4. Possible dual role of GSTP1 genotype in airway inflammation: detoxification of reactive oxygen species and/or metabolism of eicosanoids.

host genetic makeup and the external environment, predisposing high-risk individuals to respiratory symptoms and/or determining clinical outcome by altering host protection against irritant-driven oxidant stress (a key component of the airway inflammation and tissue damage underlying asthma). Furthermore, these GST can also influence synthesis of eicosanoids, critical mediators of the atopic asthmatic response. Since the effect of polymorphism in GSTP1 on activity toward lipid hydroperoxide and eicosanoid substrates is unknown, two putative mechanisms are proposed (Fig. 4). Firstly, increased catalytic efficiency toward lipid hydroperoxides in individuals with GSTP1 Val/Val would decrease the ability of these ROS products to mobilize arachidonic acid, thereby reducing synthesis of proinflammatory eicosanoids such as LTA₄-LTC₄ and PGF_{2α}. Alternatively, decreased activity toward eicosanoids in individuals with GSTP1 Val/Val would also reduce synthesis of proinflammatory eicosanoids. Further research is clearly needed to explore the details of the direct functional significance of interindividual variation in GSTP1 genes (genetically mediated or otherwise) at the airway level; in particular, how they influence the generation of particular eicosanoid metabolites. Such studies could provide new targets to be exploited in the never-ending search for asthma disease-modifying or preventing agents.

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