Pharmacogenetics of asthma

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Recent advances in the extent of knowledge regarding interindividual genetic variation in drug treatment targets and drug metabolizing enzymes has resulted in studies designed to assess the contribution of genetic variability to treatment response in a range of diseases. This review describes the current state of knowledge of genetic variability in key airway targets important in the treatment of asthma. Whilst the genes coding for some key treatment targets contain little polymorphic variation (e.g. the muscarinic M₂ and M₃ receptors) other genes whose products are important targets in the treatment of asthma contain extensive genetic variation. The best examples of the latter are the β_2 -adrenoceptor and the 5-lipoxygenase genes. Genetic variability in both of these genes may account in part for interindividual variability in treatment response. Finally, a number of key targets within the airways remain to be adequately screened for polymorphic variation.

Keywords: asthma, pharmacogenetics, polymorphism, β_2 -adrenoceptor, 5-lipoxygenase

Introduction

Bronchial asthma is a disease which is both polygenic and multifactorial in nature. Various genetic factors, coupled with environmental stimuli interact in susceptible individuals to produce the phenotype. The pathophysiology consists of chronic inflammation of the airways accompanied by a degree of airway wall remodelling. This results in decreased airway calibre. The accumulation of inflammatory cells to the airway lining, together with the expression and release of a host of pro-inflammatory agents, contribute to the development of oedema, mucus hypersecretion, and epithelial cell shedding. Complete plugging of terminal airway bronchioles may occur [1]. Stimulation of exposed subepithelial parasympathetic sensory nerve endings leads to vagally induced smooth muscle contraction, and the airways become hyper-responsive to a variety of stimuli which include allergens and irritants. Indeed, airway hyperresponsiveness is a hallmark of asthma [2].

The multifactorial and polygenic nature of asthma is best explained by a model in which there is interplay

between environmental and genetic dosage effects. According to this model, an individual having several mutations in genes which may predispose to developing asthma, only needs a small degree of environmental exposure in order to develop the disease; while an individual having few mutations needs a much greater exposure to environmental triggers in order to develop symptoms. A person having no mutations predisposing to asthma would not develop the disease, irrespective of the degree of contribution from environmental exposure [3].

The study of genetic factors which may contribute to the asthma phenotype has followed two paths. *Linkage analysis* studies, using whole or partial genome screens with polymorphic genotypic markers, have yielded information about loci and genes which are coinherited with disease markers, and which therefore might potentially contribute to the expression of the phenotype [4]. *Candidate gene* studies, have focused on revealing the contribution of selected genes, to the development of the disease (disease-causing genes), to its severity (disease-modifying genes) or to variations in its response to therapy (treatment response genes) [5–7]. Defining the extent of polymorphic variation in treatment response genes is of pharmacogenetic importance, and may eventually help to allow us to predict drug responses in particular patients from genotype profiles. A nonexhaustive list of genes in which polymorphisms have been identified that might

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potentially have pharmacogenetic implications is given in Table 1.

Precise phenotypic definition is a critical issue in the study of genetic factors which affect disease. The asthma phenotype is heterogeneous, and some features may or may not be exhibited by different patients. In order to address this problem, two main approaches have been proposed. The first involves the classification of different 'asthmas' according to precisely defined clinical and/or diagnostic criteria. The second approach, which is almost universally used, investigates intermediate phenotypes (e.g. airway hyperresponsiveness, serum IgE levels, frequency of wheezing), and attempts to identify the genetic contributions to that characteristic. This latter approach simplifies the mathematical procedures, and affords a greater degree of statistical power towards identifying the contributions of a given gene [8, 9]. This is, however, much less of a problem for pharmacogenetic studies, where the main end point of interest is response to treatment. The potential for genetic variation in the major targets for asthma treatment to contribute to variability in response is discussed below.

β_2 -adrenoceptors

β_2 -adrenoceptor agonists constitute the most important bronchodilator drugs used in the management of asthma. These drugs activate β_2 -adrenoceptors on airway smooth

muscle cells [10], and via a G-protein coupled mechanism, bring about the activation of adenylate cyclase resulting in increased intracellular cAMP (cyclic adenosine monophosphate) concentrations and a relaxation of airway tone [11]. β_2 -adrenoceptor agonists are also thought to have direct effects upon Ca^{2+} activated K^+ channels on airway smooth muscle, and thus produce a cAMP independent relaxation [12]. In the lung, β_2 -adrenoceptors are also found on vascular endothelium [13], alveolar walls [14] and cholinergic nerve ganglia [15]. Activation of these receptors is thought to bring about enhanced mucociliary function, decrease vascular permeability and modify neurotransmitter release. The drugs also offer protection against bronchoconstrictor challenge. Long-term exposure to the drugs induces both receptor desensitization and downregulation [16].

The β_2 -adrenoceptor is a 413 amino acid protein, with 7 transmembrane spanning domains, 3 extracellular and 3 intracellular loops, an extracellular amino terminus and an intracellular carboxy tail. It is a member of the family of 7-transmembrane domain G-protein coupled receptors, and the product of a 1242 base intronless gene located on chromosome 5q31.32 [17]. The main transcriptional regulatory activity for the β_2 -adrenoceptor gene lies immediately 5' to the start codon. This region also contains the coding sequence for a 19 amino acid protein, called the Beta Upstream Peptide (BUP), which is thought to influence β_2 -adrenoceptor gene expression at a translational level.

Table 1 Non-exhaustive list of genes for which polymorphic variation could potentially have pharmacogenetic implications in asthma.

Gene	Chromosomal location	Potential treatment response affected
β_2 -adrenoceptor (ADBR2)	5q31.32	β_2 -adrenoceptor agonists (e.g. salbutamol, salmeterol)
G _{sz} (GNAS1)	20q13.2	
G _{sβ} (GNB1)	1pter-p31.2	
Adenylate cyclase	Various	
5-LOX (ALOX5)	10q11.12	5-LOX inhibitors (e.g. zileuton)
FLAP (ALOX5AP)	13q12	
LTC ₄ S (LTC4S)	5q35	CysLT ₁ antagonists (e.g. zafirlukast)
CysLT ₁	X	
M ₂ (CHRM2)	7q35.36	Muscarinic antagonists (e.g. ipratropium bromide)
M ₃ (CHRM3)	1q43.44	
GR (GRL)	5q.31	Glucocorticoids (e.g. prednisolone, beclomethasone)
NF- κ B	?	
AP-1 (JUND)	19p13.1-p12	Theophylline
PDE ₄ A (PDE4A)	19p13.2	
PDE ₄ B (PDE4B)	1p31	
PDE ₄ C (PDE4C)	19p13.1	
PDE ₄ D (PDE4D)	5q12	
A ₁ (ADORA1)	1q32.1	Montelukast, salmeterol, budesonide, theophylline
A _{2a} (ADORA2A)	22q11.12	
A _{2b} (ADORA2B)	17p12-p11.2	
CYP450	Various	

Coding region

Nine single base substitutions have to date been identified in the β_2 -adrenoceptor coding region; five of these are degenerate, and are unlikely to be functionally significant (Figure 1). Three of the other four have demonstrable *in vivo* functional effects [18, 19].

Arg→Gly16 (46A→G): The occurrence of the Gly16 form of the receptor is high; indeed higher than the Arg16 which has traditionally been considered as the wild type. The allelic frequency for this variant has been reported

to lie between 67% and 72% in different populations [19–22] (Table 2).

Studies using site directed mutagenesis and transfection to express the different forms of the β_2 -adrenoceptor in Chinese hamster fibroblast (CHW) cells, have shown the Gly16 receptor variant to undergo significantly enhanced agonist promoted downregulation compared with the wild type, following exposure to 10 μ M isoprenaline for 24 h. CHW cells do not constitutively express β_2 -adrenoceptors but contain all the necessary mechanisms for receptor-promoted signal transduction [23]. Further studies in

Figure 1 β_2 -adrenoceptor polymorphisms. Degenerate substitutions are shown in black. (Reproduced with permission from Liggett [19]).

Table 2 Allelic frequencies of known β_2 -adrenoceptor polymorphisms. (Where not explicitly given, the frequencies presented here were computed from raw data published in the referenced papers).

Polymorphism	Allelic frequency (%)	Population	Subjects	Reference
Gly16	66.2–69.3 (<i>n</i> = 595)	UK, USA	Asthmatic	[19, 22, 37]
	71.9 (<i>n</i> = 274)	UK, USA	Normal	[19, 22, 37]
Glu27	47.4–49.0 (<i>n</i> = 418)	UK, USA	Asthmatic	[19, 22, 37]
	50 (<i>n</i> = 82)	USA	Normal	[19, 22, 37]
Met34	1 (<i>n</i> = 51)	USA	Asthmatic	[22]
	0 (<i>n</i> = 56)	USA	Normal	[22]
Ile164	1.0–1.1 (<i>n</i> = 274)	USA	Asthmatic	[19, 22]
	2.4–2.5 (<i>n</i> = 260)	USA	Normal	[19, 22]

primary human airway smooth muscle (HASM) cells homozygous for Gly16, produced similar results [21].

Gln→Glu27 (79C→G): This polymorphism confers on the receptor a strong resistance towards both agonist-promoted *desensitization* and *downregulation*. Approximately 60-fold greater concentrations of isoprenaline were required to desensitize the homozygous Glu27 variant to the same extent as the homozygous Gln27 form in a primary HASM cell system [21]. While this desensitization protection mechanism has not yet been elucidated, the Glu27 induced altered down-regulation of receptor number appears to be due to a modified susceptibility to receptor protein degradation rather than to effects on *de novo* synthesis. Work carried out using site-directed mutagenesis to generate and express Gly16/Glu27 double mutant receptors, showed the Gly16 effects to be dominant over Glu27. These receptors underwent greater agonist-promoted downregulation than did wild type β_2 -adrenoceptors [23].

Thr→Ile164 (491C→T): This polymorphism is rare and therefore studies in nontransfected cell systems carrying the homozygous mutation have proven to be difficult. Transfected CHW cell studies have however, shown the Ile164 receptor to bind isoprenaline, adrenaline and nor-adrenaline with 4-fold lower affinity than the wild type Thr164 form [24]. This probably occurs due to perturbation of the hydrogen bond normally formed between adjacent Ser165 and the β -hydroxyl group of catecholamines. Binding studies with the non β -hydroxyl containing agonists dopamine and dobutamine, showed similar ligand affinities for both the Ile164 and the Thr164 forms of the receptor. A second dysfunctionality observed in the Ile164 variant, is its reduced ability to mediate basal activation of adenylate cyclase, in the absence of agonist. This observation implies the existence of a second mechanism by which this variant transduces signal less efficiently. It has been suggested that Ile164 substitution results in uncoupling of the normal basal β_2 -adrenoceptor–G protein interaction [22].

Val→Met34 (100G→A): This polymorphism is extremely rare. Agonist binding and functional coupling profiles of the Met34 from the receptor in transfected CHW cells, are similar to those of the wild type receptor. Studies have to date been unable to identify a Met34-induced receptor dysfunctionality [22].

BanI RFLP (523C→A): A degenerate C→A substitution at nucleic acid residue 523 has been reported by a Japanese group. This polymorphism can be genotyped using BanI restriction fragment length polymorphism (RFLP) analysis, and has been associated with a higher incidence of asthma and a lower response to inhaled salbutamol [25]. Since this polymorphism does not introduce an amino acid change in the protein, it appears likely that these associations could have been influenced by linkage

disequilibrium with other polymorphisms within the β_2 -adrenoceptor locus [26].

Promoter region

The β_2 -adrenoceptor 5' flanking region contains motifs for several regulatory elements, including CRE (cAMP response element), NF-IL6 (nuclear factor IL-6), AP2 (activator protein-2) and a steroid binding hexamer, besides carrying the coding sequence for the BUP.

A total of 8 polymorphisms within the 1470 bp upstream from the start codon of the β_2 -adrenoceptor gene have been identified. One (–47T→C) results in a nonconservative amino acid change (Cys→Arg) in the upstream leader peptide. The rest are at positions –20T→C, –367T→C, –468C→G, –654G→A, –1023G→A, –1343A→G and –1429T→A (Table 3). All the polymorphisms are common, and have allelic frequencies that lie between 33% and 67% in the general population. Due to their close proximity, these polymorphisms are in strong linkage disequilibrium with each other. This results in specific combinations being represented more frequently than would be expected by chance. BUP polymorphisms might potentially alter translational control of the β_2 -adrenoceptor, since BUP has been recognized to be a translational inhibitor. The other polymorphisms might potentially affect β_2 -adrenoceptor expression at a transcriptional level. Luciferase reporter studies carried out in COS-7 cells, using the most common mutant haplotype (–20C, –47C, –367C, –468G) and the wild type (–20T, –47T, –367T, –468G) revealed a small but significant decrease in promoter activity of the mutant haplotype [27]. However, recent studies in human airway smooth muscle suggest these data may not hold true in all cell types

Table 3 Polymorphisms identified within the 5' flanking region of the human β_2 -adrenoceptor gene. (reproduced with permission from Scott *et al.* [27]).

bp upstream from ATG	Base change	Putative regulatory site
–20	T→C	–
–47	T→C	Non-conservative amino acid change in 5' leader peptide (BUP: Cys→Arg)
–367	T→C	Creates 7/8 bases of a consensus AP2 site and is 7 bp 3' of an overlapping AP2/Sp1 site
–468	C→G	–
–654	G→A	p53 site
–1023	G→A	2 bp 3' of an NF-IL6 site
–1343	A→G	–
–1429	T→A	3 bp 3' of steroid binding hexamer

[28]. One probable explanation for these and other data is that the true effect of polymorphisms across both the 5'UTR and the coding region of this gene is dependent upon the extended haplotype rather than individual SNPs [29].

Clinical relevance

The contribution of β_2 -adrenoceptor polymorphisms to asthma appears to be of a disease modifying rather than a disease causing nature. Most studies have shown weak or no association between β_2 -adrenoceptor polymorphisms and the presence of asthma *per se*, or phenotypic markers such as bronchial responsiveness to methacholine, atopy and wheeze frequency [30].

Asthmatic patients carrying the downregulating Gly16 β_2 -adrenoceptor are 6 times more likely to suffer from nocturnal symptoms [31] and appear to have a higher degree of airway reactivity to histamine [32]. Homozygous Gly16 adult asthmatics have also been shown to exhibit a higher loss in positive FEV₁ or FEF_{25–75} responses following formoterol treatment (24 μ g twice daily for 4 weeks) than Arg16 homozygotes [33]. Likewise, Arg16 adult homozygotes show a higher and more rapid salbutamol-evoked FEV₁ response [34] while Arg16 asthmatic children are 5.3 fold more likely to show a positive response to salbutamol treatment than mutant Gly16 homozygotes [35]. However, other authors have reported *in vivo* findings which are inconsistent with what might be expected from *in vitro* work. Hancox *et al.* could not identify any association between Gly16 and any enhanced deterioration of asthma over Arg16 patients [36]. Additionally, Gly16 appeared to *protect* against BHR during fenoterol treatment, although earlier *in vitro* work had shown Gly16 receptors to exhibit enhanced down-regulation in response to β_2 -adrenoceptor agonists [37]. Later studies found no influence of the position 16 or 27 polymorphisms on bronchoprotective desensitization in asthmatic patients on formoterol or terbutaline treatment [20, 37].

Glu27-induced resistance to receptor downregulation has been reported to decrease methacholine reactivity four-fold in homozygous patients [38]. Gln27 has been associated with elevated serum IgE levels in asthmatic families [39], and with an increased prevalence of asthma in children [40]. The effect on serum IgE could potentially either be due to decreased β_2 -adrenoceptor mediated anti-inflammatory effects, or to linkage disequilibrium between amino acid 27 and another IgE control locus nearby on chromosome 5q31 [39]. No effect of the codon 27 polymorphism on treatment response has been reported to date.

Weir *et al.* studied various haplotypes at the 16, 27 and 164 amino acid positions, and identified the Gly16/Gln27

combination to be more prevalent in moderate than mild asthmatics [41]. This haplotype, which would be expected to downregulate the receptor most, is also associated with a higher prevalence of bronchial hyperresponsiveness [42]. Clinical data on the Thr-Ile164 and Val-Met34 polymorphisms are unavailable, since researchers have been unable to obtain adequate patient numbers carrying these genotypes in order to facilitate such studies. However, the true genetic contribution to clinical responsiveness would be predicted to be related to the complete extended haplotype of an individual across this region. Preliminary data suggesting that at least bronchodilator responses may be predicted by haplotype have recently been published [29].

Knowledge of the β_2 -adrenoceptor genotype of asthmatic patients may be useful in order to predict response to bronchodilator therapy, and therefore to provide prescribers with additional knowledge which will enable them to tailor disease management programs for individual patients. It is also interesting to note that the activity of catechol-O-methyltransferase (COMT, E.C. 2.1.1.6), one of the enzymes responsible for the metabolic degradation of catecholamines and derivatives, is influenced by a common genetic polymorphism. Within the general population, about 25% of subjects are homozygous for low COMT activity [43]. It might therefore be expected that in the past when patients were given older COMT-sensitive bronchodilators, such as isoprenaline or isoetharine, altered responses to treatment may have resulted due to the prolonged pharmacokinetic half-life in individuals with low COMT activity.

5-lipoxygenase pathway

Leukotrienes constitute a family of polyunsaturated eicosatetraenoic acids that have been shown to exert a role as mediators of inflammation and asthma pathology [44, 45]. They are derived from arachidonic acid, via an enzymatic pathway for which 5-lipoxygenase, together with its constitutively expressed activating protein FLAP (5-lipoxygenase activating protein) is largely responsible [46] (Figure 2). Leukotrienes A₄, C₄, D₄, E₄ but not B₄, all contain cysteine and are known as cysteine leukotrienes. In asthma, they are released into the airways by pro-inflammatory cells including eosinophils, neutrophils and mast cells. Cysteine leukotrienes bind to specific receptors (primarily CysLT₁) and exert effects which include airway smooth muscle contraction, plasma extravasation and mucus hypersecretion [47].

LTC₄ synthase, the enzyme responsible for the production of LTC₄, also exerts an influential role on the 5-LOX pathway. Higher levels of the enzyme have been found in bronchial biopsy specimens from aspirin sensitive than aspirin tolerant asthmatics. This has been

Figure 2 The lipoxygenase pathway of leukotriene synthesis. HPETE: hydroperoxyeicosatetraenoic acid; LT: leukotriene; 5-LOX: 5-lipoxygenase; FLAP: 5-lipoxygenase activating protein; LTC₄S: leukotriene C₄ synthase.

associated with an increased LTC₄ concentration in bronchoalveolar lavage fluid, and higher numbers of LTC₄ producing cells (predominantly eosinophils) [48].

Drugs which inhibit 5-LOX (e.g. zileuton) or block CysLT₁ receptors (e.g. zafirlukast, montelukast, pranlukast) are the latest addition to the available antiasthma drugs, and they have a proven clinical efficacy in relieving symptoms [49]. The study of the genetic factors which may affect therapy with these drugs, has until now focused on the 5-LOX enzyme and LTC₄ synthase.

The 5-LOX gene (ALOX5) is located on chromosome 10q11.12. It contains 14 exons spread over more than 85 kilobases of DNA [50]. The 5' flanking region has promoter activity and is devoid of TATA or CCAAT boxes. It contains consensus sequences for several transcription factors, including Sp1, Sp3, Egr1, Egr2, NF- κ B, GATA, Myb and AP family members [51]. In particular, the 5-LOX promoter is the only known promoter to date, which contains a series of five tandem binding motifs for Sp1/Egr1 [52]. These were found to be necessary for promoter activity in a chloramphenicol acetyl transferase (CAT) reporter assay in HeLa and HL-60 cells [51]. Three functionally relevant polymorphisms have been identified within the promoter region of 5-LOX. Two were deletion polymorphisms, which resulted in the loss of one or two Sp1/Egr1 motifs (GGGCGG) from the 5 tandem repeats. The third was a 6-bp addition of an extra Sp1/Egr1 motif. These polymorphisms are common, with 35% of the population carrying one variant allele at this locus. All the three mutations showed a small decrease (25–30%) in promoter activity in a CAT-reporter assay using HeLa cells. Mobility shift studies with Sp1 and Egr1 showed that the variants displayed decreased transcription factor binding compared with the wild type.

Co-transfection of promoter-CAT reporter constructs with an expression construct for either Sp1 or Egr1 in *Drosophila* SL2 cells (Schneider cells), which do not naturally express Sp1 or Egr1, produced different CAT responses, which were in direct proportion to the number of Sp1/Egr1 consensus sequences in the promoter. The 6 bp addition mutation resulted in higher promoter activity than the wild type. This gradation in response was not observed in HeLa cells, and suggests that the functional effect of these polymorphisms depends on the cell types used [52, 53].

LTC₄ synthase gene

The gene for LTC₄ synthase (LTC₄S) is located on chromosome 5q35, in close proximity to other candidate genes for asthma. The promoter region lacks TATA or CAAT motifs, but contains consensus sequences for numerous transcription factors [54]. Sanak *et al.* (1997) identified an A→C substitution at position –444 (c.f. ATG) which resulted in an additional core motif for AP-2 (CCCG). The polymorphisms showed an association with aspirin induced asthma and could potentially contribute to increased LTC₄ in the airway [55]. As with several of the other polymorphisms described in this review, significant variability in the prevalence of this polymorphism has been observed in different ethnic groups.

CysLT₁ and CysLT₂ receptors

The human CysLT₁ and CysLT₂ receptors have been recently cloned and characterized [56, 57]. Both are members of the G-protein coupled receptor family, and hold 38% amino acid identity [57]. CysLT₁ is a 337 amino acid protein, which maps to chromosome X [56], while CysLT₂ is a 346 amino acid protein which maps to chromosomal region 13q14 [57]. The common leukotriene receptor antagonists currently marketed for the management of asthma (montelukast, zafirlukast, pranlukast), selectively bind to the CysLT₁ subtype, and are potent competitors for LTD₄, LTE₄ and LTC₄ [56]. Polymorphic variation within this receptor may be postulated to cause two effects. Firstly, the binding of endogenous leukotrienes may be affected, thus contributing to a modified asthma phenotype. Secondly, the binding affinity of leukotriene receptor antagonist drugs may be modified, thus altering the efficacy of these agents in this group of patients. Similar changes may be brought about by receptor polymorphisms which have no effect on binding affinities, but which influence the signal transduction pathways. There are to date no reported studies of mutation screening of this receptor.

Clinical relevance

Drazen *et al.* [58] addressed the question of whether asthmatics carrying the various 5-LOX promoter genotypes show altered treatment responses to a 5-LOX inhibitor drug. A study using ABT-761, a 5-LOX inhibitor derivative of zileuton, in 114 asthmatic patients, at a dose of 300 mg day⁻¹ for 84 days, demonstrated significant genotype dependent variations in treatment responses (Table 4).

It appears that the highest response to ABT-761 treatment in terms of improved pulmonary function, occurs in patients who are heterozygous or homozygous for the wild type allele at the 5-LOX promoter locus. In contrast, patients who are mutant at both alleles do not benefit from anti-5-LOX treatment, suggesting that the 5-LOX pathway is not a significant contributing factor to their asthma pathology, presumably due to lower levels of 5-LOX activity in their airways [58].

The clinical relevance of the LTCS -444A→C promoter polymorphism is not clear. It could however, be a potential risk factor for adverse reactions to nonsteroidal analgesics in asthma, since it may alter the expression pattern of the enzyme [55, 59].

Glucocorticoid receptor

Glucocorticoids (GC) have today been established as the mainstay of asthma management. They are the most potent anti-inflammatory agents available for the treatment of asthma.

Glucocorticoids act by binding to a cytoplasmic receptor (GR), which subsequently enters the nucleus and acts as a positive or negative transcriptional regulator. Through this mechanism, the transcription of various pro-inflammatory proteins, including cytokines and iNOS is decreased, while there is transcriptional upregulation of anti-inflammatory proteins, such as lipocortins [60, 61].

Besides altering the transcription of pro-inflammatory genes, GCs have also been shown to increase the transcription of β_2 -adrenoceptors in human lung [62], as well as decrease the expression of muscarinic M₂ and M₃

receptors in canine airway smooth muscle [63, 65]. Both these effects help to shift the neural influence on the airways away from vagally mediated bronchoconstriction towards sympathetically mediated relaxation of smooth muscle. Furthermore, the positive effect of glucocorticoids on β_2 -adrenoceptor expression opposes the receptor downregulation which may occur following long-term frequent use of β_2 -adrenoceptor agonist drugs.

Notwithstanding the proven clinical efficacy of GCs in asthma, there remain a subset of patients who are GC-resistant, and in whom management of the condition is rendered difficult [65]. Studies have shown that the GRs in corticosteroid-resistant asthmatics exhibit a lower interaction with activator protein-1 (AP-1), and this effect is accompanied by raised levels of AP-1 [66]. Mathieu *et al.* [67] have shown that overexpression of the GR gene in A549 human lung epithelial cells, using a GR expression vector, resulted in repression of both nuclear factor kappa-B (NF- κ B) and AP-1 activities in the absence of drug. This effect was increased following the addition of dexamethasone. AP-1 and NF- κ B repression was also observed following overexpression of a GR ligand-binding mutant in the same cell line [67]. These results are in contrast to other studies carried out in different cells, which have shown GR-mediated AP-1 and NF- κ B repression to be strictly hormone dependent [68, 69]. The mechanism for this hormone-independent, GR-mediated repression possibly involves physical association between ligand-free GR and AP-1 or NF- κ B. These findings suggest that overexpression of the GR *in vivo* may be a potentially useful approach to gene therapy, especially as a complimentary or alternative treatment for GC resistant asthmatics [67].

GR polymorphisms

The phenotypic contribution in asthmatics of currently identified polymorphisms in the human GR, is currently not well defined. A single amino acid substitution at position 641 (Val→Asp) resulted in a three-fold lower binding affinity for dexamethasone when expressed in COS-7 cells [70], while a Val→Ile amino acid substitution at position 729 has been shown to result in a four fold decrease in dexamethasone activity [71]. A third polymorphism resulting in a Asn→Ser at codon 363, appears to result in a higher sensitivity to exogenously administered glucocorticoids in healthy elderly individuals, with respect to cortisol suppression. Subjects carrying this polymorphism tend to have a higher body mass index and a lower bone mineral density compared to wild type individuals [72]. Studies carried out specifically in corticosteroid-resistant asthmatics have however, failed to link glucocorticoid resistance to polymorphic variation within the GR. Mutation screening of the human GR

Table 4 Effects of ABT-761 on FEV₁ improvement in asthmatics carrying 5-LOX promoter polymorphisms. (a) $P < 0.0006$; (b) $P < 0.0001$.

Genotype	n	Average percentage improvement in FEV ₁ at day 84
Homozygous wild	64	18.8 ± 3.6
Heterozygous	40	23.3 ± 6.0
Homozygous mutant	10	-1.29 ± 2.9

(a) (b)

in corticosteroid-resistant asthmatics by single stranded conformation polymorphism (SSCP) analysis [73] or chemical mutational analysis [74] failed to associate any polymorphisms with resistance, although Koper *et al.* identified five novel polymorphisms in the GR gene [73]. Glucocorticoid resistance, therefore does not appear to be caused by polymorphic variation within the human GR. However it may be postulated that asthmatic patients carrying the 641Val→Asp or the 729Val→Ile GR variants may exhibit a decreased clinical response to glucocorticoid administration than the respective wild-type individuals.

Phosphodiesterase

Phosphodiesterases are responsible for the degradation of cAMP, and therefore act to oppose cAMP-mediated relaxation of bronchial smooth muscle. At least seven different phosphodiesterase enzyme families are expressed in humans, of which type 4 (PDE₄) represents the predominant cAMP hydrolysing activity in human airway smooth muscle, eosinophils and neutrophils [75]. Four human genes have been identified which code for PDE₄ (PDE_{4A}, PDE_{4B}, PDE_{4C}, PDE_{4D}). The phosphodiesterase genes are often complex with many introns and splice variants; for example at least five potential PDE₄ variants may be generated from the PDE_{4D} gene [76].

Alterations in the activity of PDE₄ enzymes or variations in their degree of expression may be envisaged to modify asthma disease status by altering the determinants of airway tone. Augmentation of PDE₄ activity might be expected to decrease β_2 -adrenoceptor agonist response, by degrading *de novo* β_2 -adrenoceptor agonist mediated cAMP. Variations in enzyme activity might also alter the response to theophylline, although it is not yet clear whether the *in vitro* phosphodiesterase inhibitory action of theophylline also occurs *in vivo* [77, 78]. Indeed, the development of 'second generation theophyllines' which specifically inhibit PDE₄ enzymes *in vivo*, is underway with phase III clinical trials of PDE₄ selective inhibitors currently in progress [79]. Alterations in phosphodiesterase enzyme activities could be due to promoter variants which result in different expression profiles, or to coding-region polymorphisms which directly affect the enzyme functionality. Database searches suggest that phosphodiesterase genes may contain a number of polymorphisms; however, there are currently no available data on the mutation screening of phosphodiesterase genes in asthmatics.

Cytochrome P450

Mammalian cytochrome P450 (CYP450) comprises 17 families of heme-containing enzymes which have a prime metabolizing role. Originally discovered in rat

liver microsomes, CYP450s are today known to be responsible for the metabolic degradation of various drugs, as well as to exert gene regulatory actions by 'switching on' inducible genes via chemical signals. Several genes code for the different P450 enzymes, and substantial polymorphic variation has been shown to exist [80, 81].

Antiasthma drugs which are subject to CYP450 metabolism in humans, would be expected to display altered pharmacokinetic profiles in patients carrying the appropriate CYP450 gene variants. Montelukast is sulf-oxidated and 21-hydroxylated by the CYP3A4 P450 isoform, while CYP2C9 mediates methyl-hydroxylation of the drug [82]. Salmeterol and budesonide are likewise oxidized by CYP3A [83, 84], while CYP1A2 is the major enzyme which metabolizes theophylline at therapeutic concentrations [85]. Functional polymorphisms affecting the genes coding for these P450 isoforms might be important determinants of responses to these drug treatments in asthmatics.

CYP450 polymorphisms

Polymorphic variation within the genes for several CYP450 enzymes have been described, but their functional significance with respect to response to antiasthma drugs has not been investigated. As far as the CYP450 isoforms important for the metabolism of currently used asthma treatment is concerned the following is known. At least five polymorphisms in the CYP2C9 isoform have been identified (144Arg→Cys, 358Tyr→Cys, 359Ile→Leu, 359Ile→Thr, 417Gly→Asp) [85, 87]. Studies on diclofenac metabolism have shown the heterozygous Leu359 variant to result in a K_m value which is eight fold higher than that observed with homozygous Ile359, and 4–5 times higher than heterozygous Thr359. V_{max} values were also highest for Leu359 carrier status [87].

Phenotypic variations in CYP1A2 activity have been identified [88], but researchers have been unable to identify polymorphic variation within the exons of the CYP1A2 gene [89]. Polymorphisms have however, been identified within the 5' flanking region and intron 1 [89–91]. The intron 1 polymorphism (734C→A) may confer a higher inducibility of the enzyme [92], while one of the 5' polymorphisms (–2964G→A) appears to cause decreased enzyme activity [93] in caffeine metabolism studies in humans but does not affect steady state plasma concentrations of haloperidol [94].

With respect to the CYP3A gene, one polymorphism has been identified in the 5' flanking region (–292A→G). This does not appear to affect the metabolism of erythromycin or nifedipine [95], but GG homozygotes exhibited a 30% decrease in the systemic clearance of midazolam [96].

It is reasonable to expect the functional effects of polymorphic variation in CYP450 enzymes to be exhibited in most drugs metabolized by the specific variant. Therefore, until further results are available, it can be postulated that CYP450 polymorphisms may contribute to variations in therapeutic responses to anti-asthmatic drugs which are metabolized by the respective variant. Fast metabolizers may show decreased treatment responses while slow metabolizers may be more likely to experience adverse effects.

Muscarinic receptors

Polymorphic variation within muscarinic M_2 and M_3 receptors could potentially alter treatment responses to anticholinergic agents, such as ipratropium bromide. Within the airways, muscarinic M_2 receptors are found postjunctionally on smooth muscle cells and prejunctionally on parasympathetic terminal nerve endings. The latter M_2 receptors, function as autoreceptors, and act to mediate negative feedback control on acetylcholine release [97, 98]. Acute stimulation of postjunctional M_2 receptors act to inhibit adenylate cyclase activation via coupling to an inhibitory G-protein G_i , and they therefore decrease the degree of cAMP-induced airway smooth muscle relaxation, whereas chronic stimulation results in sensitization of adenylate cyclase [99]. M_3 receptors are found on airway smooth muscle and mucus glands, and they mediate vagally induced smooth muscle contraction and mucus hypersecretion [100]. The currently available antimuscarinic agents used for asthma, do not differentiate between the receptor subtypes. Mutation screening of the M_2 receptor gene by single stranded conformation polymorphism analysis has only yielded two degenerate polymorphisms in the coding region (1197T→C, Thr→Thr; 976A→C, Arg→Arg; c.f. ATG) and a common single base substitution in the 3' noncoding region (1696T→A), none of which is likely to be functionally relevant.

Pharmacogenetics and asthma treatment

The understanding of genetic factors which contribute to disease status is an important step towards identifying new therapeutic targets, and optimizing currently available therapy. Polymorphic variation within appropriate genes may potentially explain variations in treatment responses in terms of (i) efficacy and potency, (ii) half-life, (iii) composition of metabolites (drugs which are metabolized by more than one pathway, may show altered metabolic profiles if one pathway is affected by a treatment response polymorphism), (iv) duration of action (e.g. any polymorphic variation which affects binding of the long

acting β_2 -adrenoceptor agonists to the exosite), as well as (v) the development of adverse drug reactions.

The importance of recognizing functional polymorphisms in treatment response genes lies in their potential contribution towards the development of individualized patient-orientated treatment protocols. The identification and screening of appropriate gene targets may provide us with the answers to the frequently observed wide interpatient variations in clinical responses to the same treatment regimens. Selected DNA regions from large numbers of individuals may be genotyped for polymorphic markers at high resolution, and linkage analysis studies subsequently used to identify areas which show positive linkage to treatment response characteristics (e.g. slow metabolism, the appearance of a specific adverse drug reaction, etc.). Sequencing of these 'hotspot' regions would yield information about any polymorphic variation, which would then be studied from a functional aspect. This approach may eventually allow clinicians to predict the response characteristics to a drug and the development of adverse reactions, from a patient's genotype. Treatment protocols may thus be optimized on an individual basis, to maximize the benefit to risk ratio, and attain better disease control. Work is already underway to map large regions of 'hotspot' DNA for single nucleotide polymorphisms (SNPs), and use this information to develop genotyping assays that would predict specific treatment response outcomes [101].

Conclusions

A background degree of polymorphic variation exists throughout the whole human genome. Most of these polymorphisms occur in regions of DNA which are believed to be functionless, but a substantial amount occur in recognized coding or regulatory regions. These polymorphisms may or may not result in altered protein function and expression. Degenerate polymorphisms in coding regions are unlikely to be associated with functional changes, unless these are in linkage disequilibrium with a functionally relevant polymorphism elsewhere. Non-degenerate coding polymorphisms and polymorphisms in regulatory regions are more likely to give rise to altered protein function and/or expression, and thus contribute to phenotypic variation. This is exemplified by the β_2 -adrenoceptor coding region and promoter region polymorphisms.

Polymorphism-induced functional alteration is often first demonstrated in cell culture models. Results obtained from such systems cannot be automatically extrapolated to patients. In a cell culture, the gene being studied is usually placed under the control of a different promoter, and is often expressed in cells which are different from those in which its functional effects would be expressed in the

human. Moreover, cell systems consist of healthy cells, and they do not necessarily emulate the disease condition. Only after having studied genetic variants in human patient models, can one obtain evidence of their actual relevance to phenotype. A coding region substitution in a drug receptor gene may alter the receptor affinity for drug in binding assays, but unless the effect also alters treatment response in human patient studies, it is difficult to assign significant relevance to the polymorphism.

The discovery of a novel gene variant of high allelic frequency, may warrant modifications of standard treatment protocols in order to optimize management in a greater number of patients. Identification of a rare pharmacogenetic variant, which however, poses serious therapeutic implications, would allow for better management of selected patients who might otherwise be classified as difficult to treat. Such an example would be the potential implications of carrying a homozygous Thr-Ile164 β_2 -adrenoceptor genotype. Such patients would be expected to have only minimal or no clinical response to β_2 -adrenoceptor agonist treatment. However this polymorphism is extremely rare, and to date, researchers have only identified heterozygous patients.

At present, the magnitude of effect of known polymorphisms in asthma treatment targets suggests that routine genotyping of all patients before treatment is unlikely to be cost-effective. It is however, not difficult to speculate that as new data becomes available, and novel therapies are developed, the knowledge of patients' genotypes will be a necessary requisite in order to enable clinicians to optimize management of the disease. At present a strong argument could be made for this approach in, for example, patients commencing isoniazid treatment (influenced by acetylase status) or warfarin (influenced by CYP450 2C9 status). Genotyping of individuals for polymorphisms which may alter treatment responses in asthma is not indicated until we have further data on the effect of polymorphisms on treatment response and the cost-benefit ratio of genotyping. It is likely that at best some of the variations in treatment response would be explained by genetic factors and with the reducing cost of genotyping, it may well prove in the future to be cost-effective to screen individuals before commencing treatment.

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References

- Hogg JC. The pathology of asthma. *APMIS* 1997; **105**: 735–745.
- Page C. Bronchial hyperresponsiveness: what causes twitchy airways? *J Pharm Pharmacol* 1997; **49**(Suppl 3): 9–11.
- Hall IP. Pharmacogenetics of asthma. *Eur Respir J* 2000; **15**: 449–451.
- Hopkin JM, Cookson WO, Young RP. Asthma, atopy, and genetic linkage. *Ann NY Acad Sci* 1991; **629**: 26–30.
- Hall IP. The genetics of asthma. Candidate gene approaches: gene–environmental interactions. *Clin Exp Allergy* 1998; **28**(Suppl 1): 74–76.
- Moffatt MF, Cookson WO. Linkage and candidate gene studies in asthma. *Am J Respir Crit Care Med* 1997; **156**: S110–S112.
- Cookson W. The alliance of genes and environment in asthma and allergy. *Nature* 1999; **402**: B5–B11.
- Woolcock AJ, King G. Is there a specific phenotype for asthma? *Clin Exp Allergy* 1995; **25**(Suppl 2): 3–7.
- Neijens HJ. Determinants and regulating processes in bronchial hyperreactivity. *Lung* 1990; **168**(Suppl): 268–277.
- Hall IP, Widdop S, Townsend P, Daykin K. Control of cyclic AMP levels in primary cultures of human tracheal smooth muscle cells. *Br J Pharmacol* 1992; **107**: 422–428.
- Liggett SB, Raymond J. Pharmacology and molecular biology of adrenergic receptors. In *Catecholamines, Balliere's Clinical Endocrinology and Metabolism*, 7 Edition, ed Bouloux PM. London: Saunders, 1993: 279–306.
- Kume H, Hall IP, Washabau RJ, Takagi K, Kotlikoff MI. β -adrenergic agonists regulate KCa channels in airway smooth muscle by cAMP-dependent and -independent mechanisms. *J Clin Invest* 1994; **93**: 371–379.
- Hamid QA, Mak JC, Sheppard MN, Corrin B, Venter JC, Barnes PJ. Localization of β_2 -adrenoceptor messenger RNA in human and rat lung using *in situ* hybridization: correlation with receptor autoradiography. *Eur J Pharmacol* 1991; **206**: 133–138.
- Nijkamp FP, Engels F, Henricks PA, Van Oosterhout AJ. Mechanisms of β_2 -adrenergic receptor regulation in lungs and its implications for physiological responses. *Physiol Rev* 1992; **72**: 323–367.
- Barnes PJ. Modulation of neurotransmission in airways. *Physiol Rev* 1992; **72**: 699–729.
- Hall IP, Tattersfield A. β -agonists. In *Asthma*, eds. Clark TJ, Godfrey S, Lee T. London: Chapman & Hall, 1992: 341–365.
- Kobilka BK, Dixon RA, Frielle T, *et al.* cDNA for the human β_2 -adrenergic receptor: a protein with multiple membrane-spanning domains and encoded by a gene whose chromosomal location is shared with that of the receptor for platelet-derived growth factor. *Proc Natl Acad Sci USA* 1987; **84**: 46–50.
- Reihnsaus E, Innis M, MacIntyre N, Liggett SB. Mutations in the gene encoding for the β_2 -adrenergic receptor in normal and asthmatic subjects. *Am J Respir Cell Mol Biol* 1993; **8**: 334–339.
- Liggett SB. Polymorphisms of the β_2 -adrenergic receptor and asthma. *Am J Respir Crit Care Med* 1997; **156**: S156–S162.
- Tan S, Hall IP, Dewar J, Dow E, Lipworth B. Association between β_2 -adrenoceptor polymorphism and susceptibility to bronchodilator desensitisation in moderately severe stable asthmatics. *Lancet* 1997; **350**: 995–999.
- Green SA, Turki J, Bejarano P, Hall IP, Liggett SB. Influence of β_2 -adrenergic receptor genotypes on signal transduction

- in human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 1995; **13**: 25–33.
- 22 Green SA, Turki J, Hall IP, Liggett SB. Implications of genetic variability of human β_2 -adrenergic receptor structure. *Pulm Pharmacol* 1995; **8**: 1–10.
 - 23 Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human β_2 -adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry* 1994; **33**: 9414–9419.
 - 24 Green SA, Cole G, Jacinto M, Innis M, Liggett SB. A polymorphism of the human β_2 -adrenergic receptor within the fourth transmembrane domain alters ligand binding and functional properties of the receptor. *J Biol Chem* 1993; **268**: 23116–23121.
 - 25 Ohe M, Munakata M, Hizawa N, *et al.* β_2 -adrenergic receptor gene restriction fragment length polymorphism and bronchial asthma. *Thorax* 1995; **50**: 353–359.
 - 26 Hall IP. β_2 -adrenoceptor polymorphisms and asthma. *Monogr Allergy* 1996; **33**: 153–167.
 - 27 Scott MG, Swan C, Wheatley AP, Hall IP. Identification of novel polymorphisms within the promoter region of the human β_2 -adrenergic receptor gene. *Br J Pharmacol* 1999; **126**: 841–844.
 - 28 Drysdale C, McGraw DW, Stack C, *et al.* Complex promoter and coding region β_2 -adrenergic receptor haplotypes alter receptor expression and predict *in vivo* responsiveness. *PNAS* 2000; **97**: 10483–10488.
 - 29 Swan C, Scott MG, Hall IP. Role of the 5' CRE and 5' UTR polymorphisms in the β_2 adrenergic receptor expression in human airway smooth muscle. *Am J Resp Crit Care Med* 2000; **161**: A762.
 - 30 Dewar JC, Wheatley AP, Venn A, Morrison JF, Britton J, Hall IP. β_2 -adrenoceptor polymorphisms are in linkage disequilibrium, but are not associated with asthma in an adult population. *Clin Exp Allergy* 1998; **28**: 442–448.
 - 31 Turki J, Pak J, Green SA, Martin RJ, Liggett SB. Genetic polymorphisms of the β_2 -adrenergic receptor in nocturnal and nonnocturnal asthma. Evidence that Gly16 correlates with the nocturnal phenotype. *J Clin Invest* 1995; **95**: 1635–1641.
 - 32 Holroyd KJ, Levitt R, Dragwa C, *et al.* Evidence for β_2 -adrenergic receptor polymorphism at amino acid 16 as a risk factor for bronchial hyperresponsiveness. *Am J Respir Crit Care Med* 1995; **151**: A673.
 - 33 Aziz I, Hall IP, McFarlane LC, Lipworth BJ. β_2 -adrenoceptor regulation and bronchodilator sensitivity after regular treatment with formoterol in subjects with stable asthma. *J Allergy Clin Immunol* 1998; **101**: 337–341.
 - 34 Lima JJ, Thomason DB, Mohamed MH, Eberle LV, Self TH, Johnson JA. Impact of genetic polymorphisms of the β_2 -adrenergic receptor on albuterol bronchodilator pharmacodynamics. *Clin Pharmacol Ther* 1999; **65**: 519–525.
 - 35 Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the β_2 -adrenoceptor and response to albuterol in children with and without a history of wheezing. *J Clin Invest* 1997; **100**: 3184–3188.
 - 36 Hancox RJ, Sears MR, Taylor DR. Polymorphism of the β_2 -adrenoceptor and the response to long-term β_2 -agonist therapy in asthma. *Eur Respir J* 1998; **11**: 589–593.
 - 37 Lipworth BJ, Hall IP, Tan S, Aziz I, Coutie W. Effects of genetic polymorphism on *ex vivo* and *in vivo* function of β_2 -adrenoceptors in asthmatic patients. *Chest* 1999; **115**: 324–328.
 - 38 Hall IP, Wheatley A, Wilding P, Liggett SB. Association of Glu 27 β_2 -adrenoceptor polymorphism with lower airway reactivity in asthmatic subjects. *Lancet* 1995; **345**: 1213–1214.
 - 39 Dewar JC, Wilkinson J, Wheatley A, *et al.* The glutamine 27 β_2 -adrenoceptor polymorphism is associated with elevated IgE levels in asthmatic families. *J Allergy Clin Immunol* 1997; **100**: 261–265.
 - 40 Hopes E, McDougall C, Christie G, *et al.* Association of glutamine 27 polymorphism of β_2 -adrenoceptor with reported childhood asthma: population based study. *Br Med J* 1998; **316**: 664.
 - 41 Weir TD, Mallek N, Sandford AJ, *et al.* β_2 -Adrenergic receptor haplotypes in mild, moderate and fatal/near fatal asthma. *Am J Respir Crit Care Med* 1998; **158**: 787–791.
 - 42 D'Amato M, Vitiani LR, Petrelli G, *et al.* Association of persistent bronchial hyperresponsiveness with β_2 -adrenoceptor (ADRB2) haplotypes. A population study. *Am J Respir Crit Care Med* 1998; **158**: 1968–1973.
 - 43 Boudikova B, Szumlanski C, Maidak B, Weinshilboum R. Human liver catechol-O-methyltransferase pharmacogenetics. *Clin Pharmacol Ther* 1990; **48**: 381–389.
 - 44 Samuelsson B. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* 1983; **220**: 568–575.
 - 45 Chanarin N, Johnston SL. Leukotrienes as a target in asthma therapy. *Drugs* 1994; **47**: 12–24.
 - 46 Dixon RA, Diehl RE, Opas E, *et al.* Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis. *Nature* 1990; **343**: 282–284.
 - 47 Drazen JM. Leukotrienes in asthma and rhinitis. In *Asthma and Rhinitis*, eds. Busse W, Holgate ST. Oxford: Blackwell Scientific Publications, 1995: 838–850.
 - 48 Sampson AP, Cowburn AS, Sladek K. Profound overexpression of leukotriene C4 synthase in bronchial biopsies from aspirin intolerant asthmatic patients. *Int Arch Allergy Immunol* 1997; **113**: 355–357.
 - 49 Holgate ST, Bradding P, Sampson AP. Leukotriene antagonists and synthesis inhibitors. *J Allergy Clin Immunol* 1996; **98**: 1–13.
 - 50 Funk CD, Hoshiko S, Matsumoto T, Radmark O, Samuelsson B. Characterization of the human 5-lipoxygenase gene. *Proc Natl Acad Sci USA* 1989; **86**: 2587–2591.
 - 51 Hoshiko S, Radmark O, Samuelsson B. Characterization of the human 5-lipoxygenase gene promoter. *Proc Natl Acad Sci USA* 1990; **87**: 9073–9077.
 - 52 Silverman E, In KH, Yandava C, Drazen JM. Pharmacogenetics of the 5-lipoxygenase pathway in asthma. *Clin Exp Allergy* 1998; **28**(Suppl 5): 164–170.
 - 53 In KH, Asano K, Beier D, *et al.* Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *J Clin Invest* 1997; **99**: 1130–1137.
 - 54 Penrose JF, Spector J, Baldasaro M, *et al.* Molecular cloning of the gene for human leukotriene C4 synthase. Organization, nucleotide sequence, and chromosomal localization to 5q35. *J Biol Chem* 1996; **271**: 11356–11361.

- 55 Sanak M, Simon HU, Szczeklik A. Leukotriene C4 synthase promoter polymorphism and risk of aspirin-induced asthma [letter]. *Lancet* 1997; **350**: 1599–1600.
- 56 Lynch KR, O'Neill GP, Liu Q, *et al.* Characterization of the human cysteinyl leukotriene CysLT1 receptor. *Nature* 1999; **399**: 789–793.
- 57 Heise CE, O'Dowd BF, Figueroa DJ, *et al.* Characterization of the human cysteinyl leukotriene 2 receptor. *J Biol Chem* 2000; **275**: 30531–30536.
- 58 Drazen JM, Yandava CN, Dube L, *et al.* Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. *Nat Genet* 1999; **22**: 168–170.
- 59 Silverman ES, Drazen JM. Genetic variations in the 5-lipoxygenase core promoter. *Am J Respir Crit Care Med* 2000; **161**: S77–S80.
- 60 Barnes PJ. Mechanisms of action of glucocorticoids in asthma. *Am J Respir Crit Care Med* 1996; **154**: S21–S26.
- 61 van dV, V. Glucocorticoids: mechanisms of action and anti-inflammatory potential in asthma. *Mediators Inflamm* 1998; **7**: 229–237.
- 62 Mak JC, Nishikawa M, Barnes PJ. Glucocorticosteroids increase β_2 -adrenergic receptor transcription in human lung. *Am J Physiol* 1995; **268**: L41–L46.
- 63 Emala CW, Clancy J, Hirshman CA. Glucocorticoid treatment decreases muscarinic receptor expression in canine airway smooth muscle. *Am J Physiol* 1997; **272**: L745–L751.
- 64 Emala CW, Clancy J, Hirshman CA. *In vivo* glucocorticoids decrease expression of muscarinic receptors in airway smooth muscle. *Life Sci* 1997; **60** (13/14): 1199 (Abstract).
- 65 Szefer SJ, Leung DY. Glucocorticoid-resistant asthma: pathogenesis and clinical implications for management. *Eur Respir J* 1997; **10**: 1640–1647.
- 66 Adcock IM, Lane SJ, Brown CR, Lee TH, Barnes PJ. Abnormal glucocorticoid receptor-activator protein 1 interaction in steroid-resistant asthma. *J Exp Med* 1995; **182**: 1951–1958.
- 67 Mathieu M, Gougat C, Jaffuel D, *et al.* The glucocorticoid receptor gene as a candidate for gene therapy in asthma. *Gene Ther* 1999; **6**: 245–252.
- 68 Jonat C, Rahmsdorf HJ, Park KK, *et al.* Antitumor promotion and antiinflammation: down-modulation of AP-1 Fos activity by glucocorticoid hormone. *Cell* 1990; **62**: 1189–1204.
- 69 Scheinman RI, Gualberto A, Jewell CM, Cidowski JA, Baldwin ASJ. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. *Mol Cell Biol* 1995; **15**: 943–953.
- 70 Hurley DM, Accili D, Stratakis CA, *et al.* Point mutation causing a single amino acid substitution in the hormone binding domain of the glucocorticoid receptor in familial glucocorticoid resistance. *J Clin Invest* 1991; **87**: 680–686.
- 71 Malchoff DM, Brufsky A, Reardon G, *et al.* A mutation of the glucocorticoid receptor in primary cortisol resistance. *J Clin Invest* 1993; **91**: 1918–1925.
- 72 Huizenga NA, de Koper JW, Lange P, *et al.* A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids *in vivo*. *J Clin Endocrinol Metab* 1998; **83**: 144–151.
- 73 Koper JW, de Stolk RP, Lange P, *et al.* Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. *Hum Genet* 1997; **99**: 663–668.
- 74 Lane SJ, Arm JP, Staynov DZ, Lee TH. Chemical mutational analysis of the human glucocorticoid receptor cDNA in glucocorticoid-resistant bronchial asthma. *Am J Respir Cell Mol Biol* 1994; **11**: 42–48.
- 75 Schudt C, Tenor H, Hatzelmann A. PDE isoenzymes as targets for anti-asthma drugs. *Eur Respir J* 1995; **8**: 1179–1183.
- 76 Bolger GB, Erdogan S, Jones RE, *et al.* Characterization of five different proteins produced by alternatively spliced mRNAs from the human cAMP-specific phosphodiesterase PDE4D gene. *Biochem J* 1997; **328**: 539–548.
- 77 Polson JB, Krzanowski JJ, Goldman AL, Szentivanyi A. Inhibition of human pulmonary phosphodiesterase activity by therapeutic levels of theophylline. *Clin Exp Pharmacol Physiol* 1978; **5**: 535–539.
- 78 Bergstrand H. Phosphodiesterase inhibition and theophylline. *Eur J Respir Dis Suppl* 1980; **109**: 37–44.
- 79 Schmidt D, Dent G, Rabe KF. Selective phosphodiesterase inhibitors for the treatment of bronchial asthma and chronic obstructive pulmonary disease. *Clin Exp Allergy* 1999; **29**(Suppl 2): 99–109.
- 80 Daly AK, Fairbrother KS, Smart J. Recent advances in understanding the molecular basis of polymorphisms in genes encoding cytochrome P450 enzymes. *Toxicol Lett* 1998; **102–103**: 143–147.
- 81 Ingelman-Sundberg M, Oscarson M, McLellan RA. Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. *Trends Pharmacol Sci* 1999; **20**: 342–349.
- 82 Chiba M, Xu X, Nishime JA, Balani SK, Lin JH. Hepatic microsomal metabolism of montelukast, a potent leukotriene D4 receptor antagonist, in humans. *Drug Metab Dispos* 1997; **25**: 1022–1031.
- 83 Manchee GR, Eddershaw PJ, Ranshaw LE, *et al.* The aliphatic oxidation of salmeterol to alpha-hydroxysalmeterol in human liver microsomes is catalyzed by CYP3A. *Drug Metab Dispos* 1996; **24**: 555–559.
- 84 Jonsson G, Astrom A, Andersson P. Budesonide is metabolized by cytochrome P450 3A (CYP3A) enzymes in human liver. *Drug Metab Dispos* 1995; **23**: 137–142.
- 85 Zhang ZY, Kaminsky LS. Characterization of human cytochromes P450 involved in theophylline 8-hydroxylation. *Biochem Pharmacol* 1995; **50**: 205–211.
- 86 Kimura M, Ieiri I, Mamiya K, Urae A, Higuchi S. Genetic polymorphism of cytochrome P450s, CYP2C19, and CYP2C9 in a Japanese population. *Ther Drug Monit* 1998; **20**: 243–247.
- 87 Ieiri I, Tainaka H, Morita T, *et al.* Catalytic activity of three variants (Ile, Leu, and Thr) at amino acid residue 359 in human CYP2C9 gene and simultaneous detection using single-strand conformation polymorphism analysis. *Ther Drug Monit* 2000; **22**: 237–244.
- 88 Ou-Yang DS, Huang SL, Wang W, *et al.* Phenotypic polymorphism and gender-related differences of CYP1A2 activity in a Chinese population. *Br J Clin Pharmacol* 2000; **49**: 145–151.
- 89 Nakajima M, Yokoi T, Mizutani M, Shin S, Kadlubar FF, Kamataki T. Phenotyping of CYP1A2 in Japanese

- population by analysis of caffeine urinary metabolites: absence of mutation prescribing the phenotype in the CYP1A2 gene. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 413–421.
- 90 Chida M, Yokoi T, Fukui T, Kinoshita M, Yokota J, Kamataki T. Detection of three genetic polymorphisms in the 5'-flanking region and intron 1 of human CYP1A2 in the Japanese population. *Jpn J Cancer Res* 1999; **90**: 899–902.
 - 91 Welfare MR, Aitkin M, Bassendine MF, Daly AK. Detailed modelling of caffeine metabolism and examination of the CYP1A2 gene: lack of a polymorphism in CYP1A2 in Caucasians. *Pharmacogenetics* 1999; **9**: 367–375.
 - 92 Sachse C, Brockmoller J, Bauer S, Roots I. Functional significance of a C → A polymorphism in intron 1 of the cytochrome P450 CYP1A2 gene tested with caffeine. *Br J Clin Pharmacol* 1999; **47**: 445–449.
 - 93 Nakajima M, Yokoi T, Mizutani M, Kinoshita M, Funayama M, Kamataki T. Genetic polymorphism in the 5'-flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans. *J Biochem (Tokyo)* 1999; **125**: 803–808.
 - 94 Mihara K, Suzuki A, Kondo T, *et al.* Effect of a genetic polymorphism of CYP1A2 inducibility on the steady state plasma concentrations of haloperidol and reduced haloperidol in Japanese patients with schizophrenia. *Ther Drug Monit* 2000; **22**: 245–249.
 - 95 Ball SE, Scatina J, Kao J, *et al.* Population distribution and effects on drug metabolism of a genetic variant in the 5' promoter region of CYP3A4. *Clin Pharmacol Ther* 1999; **66**: 288–294.
 - 96 Wandel C, Witte JS, Hall JM, Stein CM, Wood AJ, Wilkinson GR. CYP3A activity in African American and European American men: population differences and functional effect of the CYP3A4*1B5'-promoter region polymorphism. *Clin Pharmacol Ther* 2000; **68**: 82–91.
 - 97 Minette PA, Barnes PJ. Prejunctional inhibitory muscarinic receptors on cholinergic nerves in human and guinea pig airways. *J Appl Physiol* 1988; **64**: 2532–2537.
 - 98 Barnes PJ. Muscarinic receptor subtypes in airways. *Life Sci* 1993; **52**: 521–527.
 - 99 Billington CK, Hall IP, Mundell SJ, *et al.* Inflammatory and contractile agents sensitize specific adenylyl cyclase isoforms in human airway smooth muscle. *Am J Respir Cell Mol Biol* 1999; **21**: 597–606.
 - 100 Mak JC, Barnes PJ. Autoradiographic visualization of muscarinic receptor subtypes in human and guinea pig lung. *Am Rev Respir Dis* 1990; **141**: 1559–1568.
 - 101 Roses AD. Pharmacogenetics and the practice of medicine. *Nature* 2000; **405**: 857–865.