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Review Article

Personalised prescribing for asthma – is pharmacogenetics the answer?

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Abstract

An individual's response to anti-asthma medication is likely to arise from a complex interaction between social, environmental and inherited factors. Studies indicate that genetic factors may account for 60–80% of the heterogeneity in treatment responsiveness in asthmatics. Identifying the genetic variants responsible may potentially lead to the development of novel treatments, improved effectiveness in the use of existing treatments and better prediction of efficacy in phase II and III trials. This article will briefly outline the current methods of identifying relevant treatment-responsive genes and their genetic variants. The pharmacogenetics of the main asthma treatment groups will then be reviewed in detail. Finally, the impact of pharmacogenetics on the pharmaceutical industry, and clinical practice in the future will be discussed.

Introduction

Nationally, the prevalence of asthma is 3–4 times higher in adults and 6 times higher in children than it was 25 years ago (Asthma Audit 2001 summary). Data from the ISAAC study (The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee 1998) has shown that the UK now has the fifth highest asthma prevalence rate in 13- to 14-year-olds worldwide. Government statistics estimate that the number of people in the UK currently treated for asthma is 5.1 million, of whom 1 in 8 are children and 1 in 13 are adults. There are 74 000 emergency admissions for asthma each year, and treatment for asthma costs the NHS an estimated £850 million per year (Asthma Audit 2001 summary). With NHS resources becoming increasingly rationed, the challenge to the pharmaceutical industry and physicians alike will be to improve the cost–efficacy balance of available drug treatments.

Asthma arises from a complex series of interactions between key environmental exposures and genetic variation within disease-susceptibility genes (Tattersfield et al 2002). Similarly, the wide degree of inter-individual variability in treatment response observed in asthmatics with similar clinical phenotypes is likely to arise from a combination of environmental and genetic factors. Clearly disease severity, exposure to environmental triggers, medication compliance and drug delivery are important predictors of treatment success. However, analysis of the repeatability (r) of treatment response, defined as the fraction of the total population variance that results from among-individual differences, provides r values of 60–80% (Drazen et al 2000). This indicates that a significant degree of heterogeneity in treatment response arises due to genetic factors. The overall heritability of treatment response in any individual will ultimately be due to a unique combination of genetic variants or polymorphisms within key target treatment-response genes.

The rapidly developing field of pharmacogenetics aims to identify these genetic variants and determine their influence on drug responsiveness. This information can then subsequently be utilised to develop individually tailored treatment programmes based upon detailed pharmacogenetic profiling.

Methods of identifying treatment-responsive genes

There are two main approaches utilised to identify genes relevant to drug responsiveness in the field of pharmacogenetics: the first is the candidate gene approach, whereby

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relevant treatment response genes are initially screened for polymorphisms. Genetic variants resulting in significant in-vitro functional effects can then be assessed clinically in traditional case–control studies. The second approach utilises linkage disequilibrium: single nucleotide polymorphisms (SNPs or snips) occur at a frequency of approximately 1 per 1000 base pairs. The number of verified SNPs in the human genome is rapidly expanding: relevant data can be found in online databases including the SNP consortium database (http://snp.cshl.org). Individuals with a relevant treatment-response gene allele will also co-inherit groups of specific variants of nearby SNP markers (haplotypes) due to the phenomenon of linkage disequilibrium. This occurs when specific combinations of alleles at different loci occur more frequently than would be expected from random association. This allows the construction of SNP-linkage disequilibrium profiles (SNP-LD), which can subsequently be used in association studies to predict relevant outcomes such as treatment response. This also means that the actual treatment-response gene need not be predicted in advance, and is particularly useful for novel agents whose mechanisms of action, metabolism or toxicity may not be clearly defined. To date, only the candidate gene approach has been utilised in the study of pharmacogenetics and asthma but with the availability of increasingly denser SNP maps, and the evolution of DNA chip technology, it is likely that the second approach will become commonly exploited. The major disadvantage with the latter approach is, however, cost, due to the large populations required for study to avoid false-positive associations being identified.

Relevant treatment-responsive genes and asthma

The treatment-responsive candidate genes relevant to asthma are shown in Table 1. To date, the majority of studies have concentrated on the following areas: β_2-adrenoceptor agonist reversibility and tolerance; steroid responsiveness; the efficacy of leukotriene antagonists; the efficacy of the theophyllines; anti-cholinergic agents; the pharmacogenetics of anti-asthma drug metabolism. These will now be discussed in detail, with descriptions of genetic variation within candidate treatment-response genes, their functional impact and likely contribution to the modulation of treatment effects.

β_2-Adrenoceptor agonist efficacy and tolerance

Drugs acting as selective β_2-adrenoceptor agonists are the main bronchodilator agents available for the treatment of asthma and are used by virtually all asthmatics as rescue medication (Anon 1997). Interaction with receptors located on airway smooth muscle cells activates adenyl cyclase via a G-protein-coupled mechanism. This leads to a rise in intracellular cAMP concentration and a subsequent relaxation of smooth muscle and airway tone (Liggett & Raymond 1993). Repeated exposure to β_2-agonists causes receptor uncoupling from its effector pathway, leading to receptor desensitisation and downregulation and the development of drug tolerance. During chronic administration of both long- and short-acting β_2-agonists, drug efficacy will be determined by inter-patient differences in the balance between receptor responsiveness and the development of tolerance, a process potentially mediated by genetic variability in receptor function.

The β_2-adrenoceptor is encoded by an intronless gene on chromosome 5q31–32. Like all members of the G-protein-coupled receptor superfamily, the β_2-adrenoceptor comprises an extracelllular amino terminus, seven transmembrane-spanning domains, three intracellular and three extracellular loops and an intracellular carboxy terminus. Genetic variation within the β_2-adrenoceptor has been well described (Reishaus et al 1993). Nine single-base substitutions have been identified in the open-reading frame (ORF) of the gene (Figure 1). Five of these are degenerate, while four result in an amino acid change to the receptor occurring at positions 16 (Arg-Gly), 27 (Gln-Glu), 34 (Val-Met) and 164 (Thr-Ile). The amino acid 34 polymorphism is extremely rare and does not result in any change to receptor function in-vitro and hence has not been studied in detail (Reishaus et al 1993). In-vitro functional studies using recombinant cell systems expressing receptor variants generated by site-directed mutagenesis have demonstrated that the other three polymorphisms significantly alter receptor function. In brief, the amino acid 164 variant is located in the fourth transmembrane spanning domain, a region critical for ligand binding. The Ile 164 mutant receptor variant displays markedly reduced affinity for catechol ligands and also reduced effector coupling to adenyl cyclase (Green et al 1993). Individuals with such polymorphisms would be predicted to show a markedly reduced response to β_2-agonists. To date, clinical studies have only identified individuals heterozygous for this polymorphism and as such its clinical effects have not been fully characterised.

In contrast, the amino acid 16 and 27 receptor variants occur commonly, and have been extensively studied. Both polymorphisms occur in the extracellular amino terminus and in-vitro studies demonstrated that the Gly 16 variant undergoes enhanced downregulation in response to β_2-agonist exposure, while the Glu 27 variant demonstrated attenuated downregulation (Green et al 1994). Both polymorphisms occur with similar frequency in asthmatic and non-asthmatic populations (Dewar et al 1998) indicating that they are unlikely to be the cause of asthma per-se, but there is some evidence to suggest that they may modify the asthmatic phenotype. The Glu 27 variant has been suggested to attenuate airway hyperresponsiveness to methacholine (Hall et al 1995) while the Gln 27 allele has been associated with increased levels of basal IgE (Dewar et al 1997) and with self-reported asthma in children (Hopes et al 1998); at loci 16, the Gly 16 variant has been associated with asthma severity in one study (Reishaus et al 1993), although in general this has not been replicated (see, for example, Dewar et al 1998). The Gly 16 variant has also been shown to be commoner in patients with nocturnal asthma (Turki et al 1995).

The in-vitro data led to predictions that sub-groups of asthmatics with different genotypes may exist with

Table 1 Potential treatment-responsive candidate genes.

Biological pathway	Gene	Chromosomal location	Potential treatment response affected
β_2-adrenoceptor and effector pathway	β_2-adrenoceptor	5q31-33	β_2-agonists
	Adenylate cyclase (ADCY9)	16p13.13	
	$G_{s\alpha}$(GNAS1)	20q13.2	
	$G_{s\beta}$(GNB1)	1pter-p31.2	
	PKA		
	CREB		
Glucocorticoid receptor and effector pathway	Glucocorticoid receptor (GRL)	5q.31	Glucocorticoids (e.g. beclomethasone, prednisolone)
	NF-κB	?	
	AP-1 (JUND)	19p13.1-p12	
Muscarinic receptors	M2 (CHRM2)	7q35.36	Muscarinic antagonists (e.g. ipratroprium bromide)
	M3 (CHRM3)	1q43.44	
Cytochrome P450	CYP450	Various	Monteluklast, salmeterol, budesonide, theophylline
Phosphodiesterases	PDE_4A	19p13.2	Theophylline
	PDE_4B	1p31	
	PDE_4C	19p13.1	
	PDE_4D	5q12	
Leukotriene synthesis	5-LOX (ALOX5)	10q11.2	5-LOX inhibitors (e.g. zileuton)
	FLAP (ALOX5AP)	13q12	CysLT1 antagonists (e.g. zafirlukast)
	LTC_4S (LTC4S)	5q35	
	CysLT1	x	

markedly different responses to β_2-agonists, individuals homozygous for the Gly 16 or Gln 27 allele being expected to display increased desensitisation and tachyphylaxis. There are several studies that have investigated the pharmacogenetic impact of the loci 16 and 27 β_2-adrenoceptor polymorphisms. The data only support a role for loci 16, with loci 27 effects appearing unimportant. Three studies have suggested that individuals homozygous for the Gly 16 receptor variant show reduced treatment response indicating desensitisation following acute and chronic β_2-agonist administration (Martinez et al 1997, Tan et al 1997; Lipworth et al 1999). However, other data contrast with the treatment response predicted from the in-vitro data: Hancox et al (1998) retrospectively genotyped 64 asthmatics receiving regular fenoterol and showed that Arg 16 homozygotes demonstrated an increase in bronchial hyperresponsiveness to methacholine compared with their responsiveness when taking as-required fenoterol. These results have been

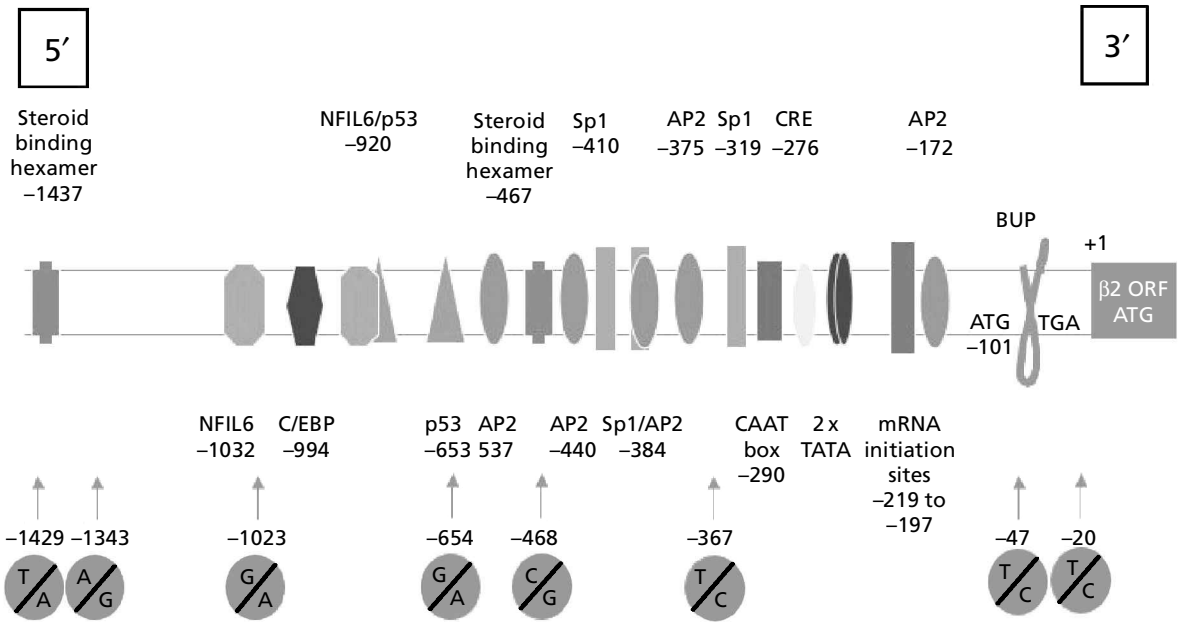

Figure 1 Schematic diagram of promoter and 5′ leader region of the β_2-adrenergic receptor.

replicated in a retrospective US study of 190 patients, where Arg 16 homozygotes demonstrated a significant decline in morning PEFR (peak expiratory flow rate) when receiving regular salbutamol, but no change when used on an as-required basis (Israel et al 2000). These findings might be explained in the context of dynamic β_2-adrenoceptor regulation (Liggett & Green 1996): the Gly 16 receptor variant is maximally downregulated by endogenous catecholamines and therefore cannot undergo further downregulation in the presence of inhaled β_2-agonists. Conversely, the attenuated basal downregulation of the Arg 16 variant allows for additional receptor downregulation by inhaled β_2-agonists, leading to the clinical phenomenon of tachyphylaxis.

Taken together, these data do suggest that genetic variation at loci 16 of the β_2-adrenoceptor can influence the response to a variety of β_2-agonists, although the clinical significance of this is not yet clear. Studies to date have been small and retrospective, and have often not taken into account the strong linkage disequilibrium between the loci 16 and 27 alleles. In addition, eight polymorphisms within the β_2-adrenoceptor promoter have now been defined (McGraw et al 1998; Scott et al 1999). One of these is located at position 19 (Arg-Cys) of the β_2-adrenoceptor upstream peptide (BUP), a key modulator of receptor translation (Figure 1). The Cys allele results in a two-fold increase in receptor expression. Drysdale et al (2000) have recently defined twelve complex promoter and coding region haplotypes, six of which occur commonly in Caucasian populations. In-vitro evidence indicates that different haplotypes confer different receptor behaviour, and preliminary evidence suggests that haplotype pairs alter response to salbutamol in asthmatics (Drysdale et al 2000). In contrast, we found no association between levels of β_2-adrenoceptor expression or cyclic AMP responses in circulating peripheral blood mononuclear cells and different haplotypes (Lipworth et al 2002). In view of these data, a definitive, large-scale prospective study assessing the effects of β_2-adrenoceptor haplotypes on the response to β_2-agonists is required before the stratification of treatment according to genotype at a population level can be evaluated.

Finally, in addition to genetic variants within the β_2-adrenoceptor itself, it is plausible that polymorphisms within other key downstream elements may be important pharmacogenetically. To date, little is known about genetic variation within the genes encoding the G-protein sub-units, adenylate cyclase and enzymes critical in receptor phosphorylation – the cAMP-independent kinases (G-protein coupled receptor kinases), and protein kinase A and C. Jia et al (1999) identified a conservative polymorphism (Ile 131) in exon 5 of the G (s) alpha gene, which was associated with hypertension, suggesting that the polymorphism may be in linkage disequilibrium with an unidentified functional variant within the locus. Clearly, further study of these genes is warranted.

Steroid responsiveness

Glucocorticoids are potent anti-inflammatory agents and are the mainstay of asthma management. However, there is increasing recognition that a sub-set of patients who do not respond to glucocorticoids exists: the steroid- or glucocorticoid-resistant asthmatic (Szefler & Leung 1997). For clinical purposes, glucocorticoid resistance is defined as the failure to improve FEV1 (forced expiratory volume in one second) by 15% from baseline of $\leq$75% predicted after a trial of prednisolone (e.g. 40 mg prednisolone for 1–2 weeks) in asthmatic patients who have demonstrated 15% reversibility to an inhaled β_2-agonist.

Glucocorticoids bind to a single class of glucocorticoid receptor localised to the cytoplasm. The glucocorticoid receptor comprises an N-terminal trans-activating domain, a central DNA-binding domain and a C-terminal hormone-binding domain (reviewed by Beato et al 1995, 2000). Glucocorticoid binding to the C-terminal causes conformational changes to the receptor and dissociation from its protein complex. This exposes nuclear translocation signals resulting in rapid nuclear translocation. The dimerised receptor then binds to a recognition sequence, the glucocorticoid response element (GRE) in the 5′ upstream promoter region of steroid-responsive genes. Gene transcription is then either up- or downregulated. The mechanism for glucocorticoid resistance is not fully understood, although it has been extensively examined. In summary, the defect does not appear to be due to impaired bioavailablity of glucocorticoids (May et al 1980); the secretion of endogenous cortisol or sensitivity of the hypothalamic–pituitary–adrenal axis is not altered (Lane et al 1996); finally, no abnormalities in receptor nuclear translocation, density or binding affinity have been demonstrated (Lane & Lee 1991).

It is now clear that there is an excess of the transcription factor activator protein-1 (AP-1) activity in glucocorticoid-resistant asthma (Adcock et al 1995). Activated glucocorticoid receptors can interact with a number of transcription factors, including AP-1 and NF-κB, thereby preventing their binding to specific response elements to regulate gene transcription, a phenomenon known as transrepression. AP-1 is formed by the dimerisation of c-fos and c-jun following the dephosphorylation of Jun N-terminal kinase (JNK). Inducible AP-1 binds to responsive elements in cytokine genes leading to gene activation. An increase in c-fos gene transcription is thought to result in excess AP-1 (Lane et al 1998), hence perpetuating AP-1 mediated inflammation, so attenuating the effects of glucocorticoids by sequestration of glucocorticoid receptors within the nucleus. Examination of the gene encoding AP-1 has not yielded any polymorphisms to explain this phenomenon (Drazen et al 2000). There are a number of reports of polymorphisms within the gene encoding c-fos, but these have not been studied, to date, in asthmatic individuals (Umino et al 2000).

A number of relatively uncommon genetic variants have been described in the gene encoding the glucocorticoid receptor (Koper et al 1997, Ruiz et al 2001). A point mutation at position 641 (Val-Asp) has been shown to result in a three-fold lower binding affinity for dexamethasone when expressed in COS-7 cells (Hurley et al 1991). Conversely, the Val-Ile substitution at position 729 confers a four-fold decrease in dexamethasone activity

(Malchoff et al 1993). Higher sensitivity to exogenously administered glucocorticoids in healthy adults has been observed in association with a third polymorphism at codon 363. In addition, individuals with this polymorphism had a higher body mass index and a lower bone mineral density (Huizenga et al 1998). Despite these observations, there is no evidence to support a link between glucocorticoid receptor polymorphisms and glucocorticoid resistance. In another study, Koper et al (1997) identified 5 novel polymorphisms within the gene by single-strand conformation polymorphism analysis. None of these variants were associated with physiological or clinical parameters of glucocorticoid resistance. The contribution of glucocorticoid receptor genetic variants to the glucocorticoid-resistant asthmatic phenotype has not been studied in detail, but chemical mutational analysis of the genes failed to reveal any base-pair mismatch between the 6 glucocorticoid-sensitive and 6 glucocorticoid-resistant individuals analysed (Lane et al 1994).

Taken together, these data suggest that glucocorticoid receptor polymorphisms are unlikely to be the cause of glucocorticoid-resistant asthma. However, intuitively, it is probable that the syndrome arises due to the combined effects of an array of polymorphisms within several candidate genes within the glucocorticoid pathway, such as c-fos, AP-1 and the glucocorticoid receptor. Further work is required to dissect the molecular and genetic basis of this interesting asthmatic phenotype. In addition, the possibility that polymorphisms within the glucocorticoid receptor might give rise to increased side-effects from glucocorticoids remains to be explored.

The efficacy of leukotriene antagonists

The leukotriene receptor antagonists comprise the newest addition to the array of anti-asthma medications. The cysteinyl leukotrienes (A_4, C_4, D_4, E_4) are potent mediators of airway inflammation and bronchoconstriction (Piper 1989; Drazen et al 1992) and are synthesised when the 5-lipoxygenase (5-LOX) pathway is activated. They are derived from arachidonic acid from cell membranes (Samuelsson et al 1983) and converted to leukotrienes via a series of biochemical reactions catalysed by 4 key enzymes. The most critical of these are 5-lipoxygenase (5-LOX) and its constitutively expressed activating protein FLAP (5-lipoxygenase activating protein) (Dixon et al 1990). These two enzymes, acting in concert with cytosolic phospholipase A_2, generate the unstable intermediate leukotriene A_4 (LTA_4) (Clark et al 1991). This is then converted to LTC_4 by the terminal enzyme of the pathway, LTC_4 synthase (Bach et al 1984). Subsequently, LTC_4 is transported to the extracellular environment for further enzymatic conversion to the bioactive metabolites LTD_4 and LTE_4 (Anderson et al 1982; Lee et al 1983). The release of cysteinyl leukotrienes into asthmatic airways by pro-inflammatory cells induces airway narrowing, bronchovascular leak, mucus gland secretion and granulocyte chemotaxis (Drazen 1995). These effects are mediated predominantly via the $CysLT_1$ receptor.

The action of leukotrienes may be pharmacologically modulated by either antagonism at the receptor site or by the inhibition of biosynthesis. There are now several pharmacological agents approved for use in asthma. Zileuton is an inhibitor of 5-LOX, while zafirlukast, pranlukast and montelukast selectively bind to the cysteinyl receptor ($CysLT_1$) and are potent competitors for LTC_4, LTD_4 and LTE_4. These drugs have been shown to have a role in treating mild-to-moderate asthma and are now indicated for its management (Anon 1997).

Drazen et al (2000) estimate that up to 60% of the variation in response to leukotriene antagonists may be attributed to genetic factors. Genetic variation within the genes encoding key enzymes 5-LOX, FLAP and LTC_4 synthase, and also the $CysLT_1$ receptor, could potentially lead to a differential response to anti-leukotriene agents. To date, the study of the pharmacogenetics of these agents has focused principally on the 5-LOX enzyme and LTC_4 synthase.

$CysLT_1$ and $CysLT_2$ receptors

The human $CysLT_1$ and $CysLT_2$ receptors have only recently been cloned and characterised (Lynch et al 1999; Heise et al 2000). They are both members of the G-protein coupled receptor super family, and share 38% homology. $CysLT_1$ is encoded by a gene mapping to chromosome X (Lynch et al 1999) and is a 337 amino acid protein, while the $CysLT_2$ receptor is a 346 amino acid protein and is encoded by a gene located on chromosome 13q14 (Heise et al 2000). Genetic variation within the $CysLT_1$ receptor could potentially modify the asthmatic phenotype per-se by affecting the binding of endogenous leukotrienes. In addition, the efficacy of the $CysLT_1$ receptor antagonists (montelukast, zafirlukast and pranlukast) may be modulated by the alteration of binding affinity or signal transduction. Hence, polymorphisms within this receptor may be of clinical relevance, but to date there are no reports of amino acid variants within the $CysLT_1$ receptor.

5-LOX

The gene encoding 5-LOX is located on chromosome 10q11.12 (Funk et al 1989). Three functionally relevant mutations have been described in the promoter region (In et al 1997). These all cause alterations in the number of tandem Sp-1 and Erg-1 consensus-binding sites. Two are deletion mutations resulting in the loss of one or two Sp1/Erg-1 motifs from the five tandem repeats found in the wild-type promoter. The third is a 6-bp addition that results in an extra Sp-1/Erg-1 motif. All three mutations have been shown to result in a small decrease (25–30%) in promoter activity when reporter constructs were studied in a CAT-reporter assay using HeLa cells (In et al 1997; Silverman et al 1998). These polymorphisms occur commonly with 35% of the population carrying at least one mutant allele. Given the functional effects on promoter activity, Drazen et al (1999) have investigated their effect on the clinical efficacy of the 5-LOX inhibitor ABT-761, a derivative of Zileuton. In a randomised, placebo-controlled and double-blinded trial, asthmatic patients were treated for 12 weeks with either placebo or ABT-761,

with FEV_1 being the primary outcome measure (Drazen et al 1999). In the actively treated group, the presence of two wild-type alleles was associated with a significantly greater increase in FEV_1 than in those with no wild-type alleles. Individuals who were mutant at both alleles did not benefit from anti-5-LOX treatment, suggesting that the 5-LOX pathway is not a significant contributing factor to their asthma, presumably due to lower levels of endogenous 5-LOX activity in their airways (Drazen et al 1999).

LTC_4 synthase

Interestingly, the gene encoding LTC_4 synthase is found on chromosome 5q35, in close proximity to one of the regions linked to asthma in several linkage analysis studies (Palmer et al 2001), a region also containing the TH-2 cytokine cluster and the β_2-adrenoceptor. A 5′ flanking region polymorphism at position −444 has been identified (Sanak et al 1997). The A–C substitution leads to an additional core motif for AP-2. The effects of the two alleles have been assessed by transfection of wild-type and mutant promoter constructs into cell lines. The mutant allele was initially reported to produce a 25% increase in reporter gene transcription (Sanak et al 1999). However, others have not been able to replicate this result (Van Sambeek et al 2000).

Aspirin-induced asthma

Aspirin-induced asthma is a distinct asthmatic phenotype occurring in up to 10% of asthmatics, consisting of a triad of nasal polyps, asthma and aspirin intolerance. In these patients, aspirin and other non-steroidal anti-inflammatory drugs induce life-threatening attacks of asthma accompanied by rhinorrhoea, conjunctival congestion and facial and neck flushing. The pathophysiology of aspirin-induced asthma is not fully understood. Measurement of COX metabolites in bronchoalveolar lavage fluid in sensitive patients demonstrates diminished biosynthesis of PGE_2. LTC_4 synthase is over-expressed in bronchial biopsy specimens of patients with aspirin-induced asthma (Cowburn et al 1998), and is associated with increased levels of LTC_4 in bronchoalveolar lavage fluid (Sampson et al 1997).

The pharmacogenetics of aspirin-induced asthma has been studied in detail. Recent genetic screening of the COX-2 gene revealed several rare polymorphisms within the gene and promoter, none of which were functionally important (Fritsche et al 2001). The inducible PGE_2 synthase gene has recently been cloned (Jakobsson et al 1999) but has not undergone mutational analysis to date. Interestingly, no association has been found between the 5-LOX promoter polymorphisms and aspirin-induced asthma (In et al 1997).

Finally, the LTC_4 synthase −444 promoter variant has been studied in the aspirin-induced asthma phenotype. Sanak et al (1997) found a significant association between the −444C allele and the aspirin-induced asthma phenotype in 76 patients. However, this finding could not be replicated by Van Sambeek et al (2000) in a sample of 69 patients with aspirin-induced asthma, 33 patients with aspirin-tolerant asthma and 137 non-asthmatic individuals. Given the over-expression of LTC_4 synthase in patients with aspirin-induced asthma, it may be worth re-screening the promoter for further polymorphisms as it seems likely that this gene could play an important role in the pathogenesis of aspirin-induced asthma.

The efficacy of the theophyllines

Theophyllines are recommended for use at stage 4 of the British Thoracic Society guidelines for the management of asthma (Anon 1997). In-vitro evidence suggests that they inhibit the action of phosphodiesterases, although their mode of action in-vivo is not clear. Phosphodiesterases degrade cAMP, and therefore oppose cAMP-mediated relaxation of bronchial smooth muscle. There are at least 7 different phosphodiesterase enzyme families expressed in man. Type 4 (PDE_4) provides the major cAMP hydrolysing activity in human airway smooth muscle, eosinophils and neutrophils (Schudt et al 1995). The genes encoding the phosphodiesterases are complex with many introns and splice variants. Four genes encode PDE_4 (PDE_4 A, B, C, D), and at least 5 potential PDE_4 variants may be generated from the PDE_4 D gene (Bolger et al 1997).

Database searches suggest that the phosphodiesterase genes may contain a number of genetic variants, although there are currently no data on the mutation screening of these genes in asthmatics or other groups. Nonetheless, this may prove to be of interest clinically, insofar as genetic variation within the genes encoding PDE_4 could be of importance either by modulating airway tone or altering the effects of β_2-agonists, theophyllines or the selective PDE_4 antagonists (e.g. cilomilast and roflumilast) currently under development. Augmentation of PDE_4 activity would be predicted to reduce the response to β_2-agonists by degrading de-novo β_2-agonist-mediated cAMP. Similarly, the effectiveness of PDE_4 antagonists could be altered due to a change in basal PDE_4 activity. Recently, it has been shown that low-dose theophylline enhances histone deacetylase activity, thereby inhibiting the acetylation of core histones necessary for inflammatory gene transcription (Ito et al 2002). Genetic variation in these enzymes may also therefore be important.

Anticholinergic drugs

While anticholinergic drugs are only generally prescribed in severe asthma, they are included here for completeness. Polymorphic variation within the muscarinic M_2 and M_3 receptors could potentially alter the treatment response to anticholinergic agents such as ipratroprium bromide. Screening of the M_2 receptor by single strand conformation polymorphism (SSCP) identified two degenerate polymorphisms in the coding region (1197T-C, Thr-Thr, and 976A-C, Arg-Arg), and a common single base substitution in the 3′ non-coding region (1696T-A) (Fenech et al 2001). None of these genetic variants are likely to be of functional significance. In this study, the M_3-receptor coding region was also screened but no common polymorphic

variants were identified. This would suggest that coding region polymorphism in the M_3 receptor would be unlikely to influence response to the recently introduced M_3-selective receptor antagonist tiotropium. The genomic arrangement of the M_2 and M_3 receptor remains to be fully defined and, to date, no information on polymorphism in regulatory regions controlling transcription of these genes is available.

The pharmacogenetics of anti-asthma drug metabolism

Anti-asthma medication subject to cytochrome P450 (CYP450) metabolic degradation in man would be predicted to display altered pharmacokinetic profiles in individuals carrying functionally relevant polymorphisms. Montelukast is sulfoxidated and 21-hydroxylated by the CYP3A4 P450 isoform, while CYP2C9 mediates methyhydroxylation of the drug. There is evidence that any systemically absorbed salmeterol and budesonide is metabolised in the liver by CYP3A (Jonsson et al 1995; Manchee et al 1996), and theophylline is metabolised by CYP1A2 at therapeutic concentrations (Zhang et al 1995). Hence, functional polymorphisms within the genes encoding these P450 isoforms might be important determinants of the therapeutic response to these drugs, including the development of adverse side-effects.

Several genes encode this family of haem-containing enzymes and substantial genetic variation has been shown to exist within these genes (Daly et al 1998, Ingelman-Sundberg et al 1999). With respect to those isoforms relevant to the metabolism of the above anti-asthma drugs, the following is known.

CYP2C9 and montelukast metabolism

At least 5 polymorphisms have been identified in the CYP2C9 isoform at codons 358 (Tyr-Cys), 359 (Ile-Leu and Ile-Thr) and 417 (Gly-Asp) (Kimura et al 1998; Ieiri et al 2000). Studies on diclofenac metabolism have demonstrated an eight-fold increase in the K_m value for the heterozygous Leu359 variant compared with the homozygous Ile359 variant, and 4–5 times higher than the heterozygous Thr359.

V_{max} values were also highest for the Leu359 carrier status (Ieiri et al 2000). The effects of these polymorphisms on montelukast metabolism have not been delineated to date.

CYP1A2 and theophylline metabolism

While phenotypic variation in CYP1A2 activity has been observed, no polymorphisms have been identified in the exons of the coding region of the gene (Nakajima et al 1994). Genetic variation has been identified within the 5′ flanking region and intron 1. The intron 1 734C-A polymorphism may confer a higher inducability of the enzyme (Sachse et al 1999), while one of the 5′ variants (−296 G-A) leads to reduced caffeine metabolism in man (Nakajima et al 1994), but does not seem to affect steady-state plasma concentrations of haloperidol (Ball et al 1999). The effect of these polymorphisms on the pharmacokinetics of theophylline are not yet understood, but may be important in predicting those individuals likely to develop potentially serious drug toxicity given its narrow therapeutic window.

CYP3A and salbutamol/budesonide metabolism

Only one polymorphism has been identified in the 5′ flanking region of the gene (−292 A-G). GG homozygotes exhibit a 30% decrease in clearance of midazolam (Wandel et al 2000) but no effect has been shown on the metabolism of either erythromycin or nifedipine (Ball et al 1999). There are no data on the effect of this genetic variant on the metabolism of salmeterol or budesonide, although given the topical mode of administration of these drugs, CYP3A polymorphism is probably unimportant in determining their efficacy.

General applications of pharmacogenetics to asthma

The cost-effectiveness of new drugs is a significant concern to patients, funding bodies and governments, particularly in the advent of regulatory bodies such as NICE (National Institute for Clinical Excellence). One of the key roles of pharmacogenetics is to maximise the benefit of medicines by prescribing only to patients in whom there is a high probability of efficacy without significant risk of adverse events. Following the discussion of specific genetic variations within treatment-response genes for current asthma medications, the broader application of pharmacogenetics to future asthma treatment and research is outlined in Figure 2.

Predicting an individual's response to a given drug by pharmacogenetic profiling

Improvements in technology and reduction in unit costs allow large-scale genetic screening for relevant polymorphisms. The development of SNP-linkage disequilibrium profiles will allow the manufacture of chips containing medicine response profiles specifically designed to exclude diagnostic information, allaying concerns about indiscriminate genetic testing.

Improving the accuracy of phase II trials

Pharmacogenetic factors may play an important role in determining the results of small phase II trials. If there is an excess of a particular treatment response genotype in one group this could lead to significant underestimation or overestimation of the compound being studied. This would then result in the development of a potentially important drug being abandoned, or expensive phase III trials being undertaken in a drug that is, in fact, not significantly effective.

Increasing the safety of phase III trials

The use of SNP-LD profiles in phase II/III trials could allow the identification of drug responders and individuals with adverse responses. A subsequent phase III trial could then recruit only responders with no adverse effects, hence

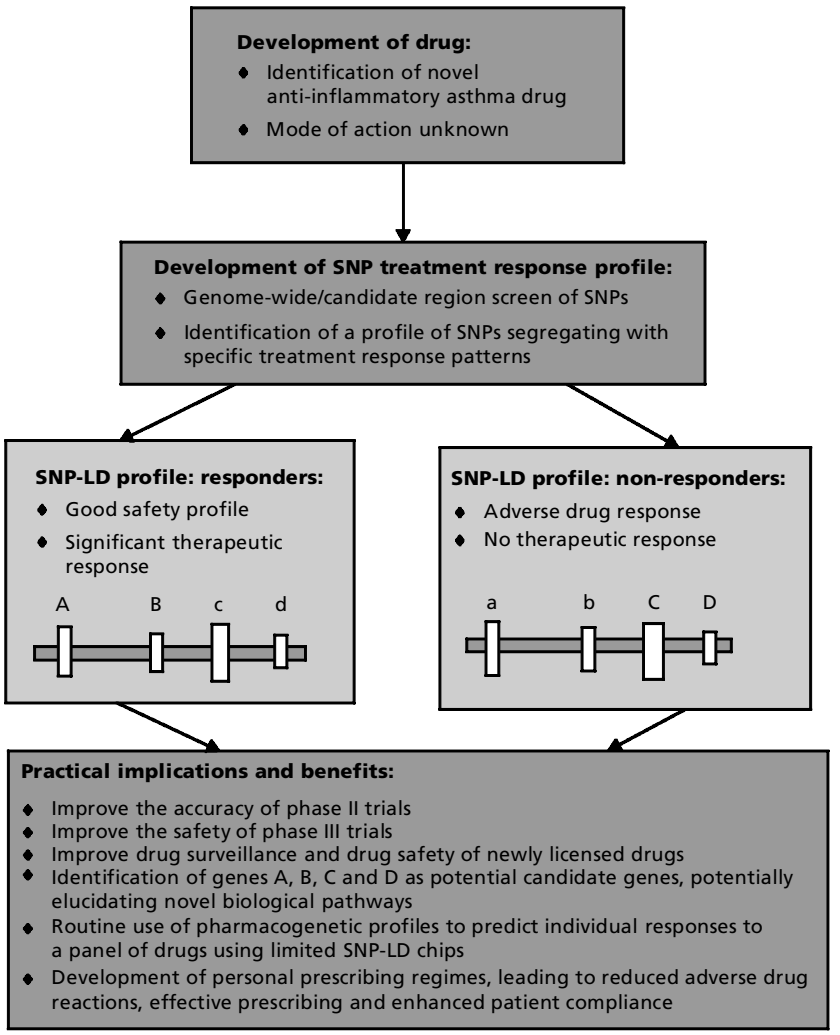

Figure 2 Scheme for the application of pharmacogenetic profiling to the development and prescribing of novel asthma drugs.

improving the efficiency and safety of the drug development process.

Improvement of drug surveillance

There is increasing recognition for the need to improve post-approval drug safety surveillance. Pharmacogenetics can be used to generate predictive adverse event profiles from the stored DNA of large numbers of patients initially taking a newly licensed drug. DNA from patients experiencing adverse events would then be compared with DNA from those patients who did not, thus acting as a control group. This adverse event profile could be combined with the treatment-response profile, hence generating a comprehensive medicine-response profile.

Discovery of novel therapeutic agents

The use of differential gene arrays evaluating gene expression in asthma, perhaps coupled with high-density genome-wide SNP screening for genes or genomic loci associated with asthma may lead to the identification of novel targets for therapy, either by identifying modulators of the asthmatic phenotype or elucidating new mechanisms of disease.

Summary

In an era where the incidence of asthma is rising in both the developed and developing world, there is an increasing need to improve the safety and efficacy of drug treatments, and to develop novel therapeutic agents. Genetic variation within treatment-response genes and drug metabolising genes may account for up to 80% of the variability observed in response to current asthma drugs. The science of pharmacogenetics combined with pharmacogenomic approaches is rapidly developing, and it is likely that in the future the technology will exist for physicians to select optimal asthma medications and dosages using a panel of disease-specific genotypes. This will allow the

identification of subsets of patients who respond well and subsets of patients who are destined to fail to respond to treatment with certain medications because of individual genetic susceptibility to drug toxicity or lack of efficacy. Whether or not this approach becomes clinically useful in the future will ultimately depend upon cost-efficiency, and the willingness of the medical community to embrace the concept of pharmacogenetics.

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