

Pharmacogenetics of asthma therapeutics

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Asthma is recognised to arise from complex interactions between environmental exposure and disease-susceptibility genetic contributions.¹ Pharmacological management of the condition aims to relieve symptoms, decrease airway hyperresponsiveness, and optimize the quality of life in patients. Inter-patient variability in the clinical responses to anti-asthma drugs is a recognized factor that may confound therapeutic outcome.

Introduction

Estimates show that as much as 60.6% of interindividual statistical variance to salbutamol response may be attributable to genetic factors, while with inhaled glucocorticoids, the figure may be as high as 86.1%.² This strongly suggests that genetic factors may significantly contribute to the clinical outcomes of pharmacological

treatment. It is the challenge of pharmacogeneticists to identify these genetic determinants and study their roles.

Pharmacogenetics and pharmacogenomics

The term *pharmacogenetics* was originally coined by Friedrich Vogel in 1959³, who used it to describe the

influence of genetic factors on the response to drugs. A second term, *pharmacogenomics*, has relatively recently found its way into the literature, and although it has often been used interchangeably with the former, pharmacogenomics is better used to describe the study of the genome and its products as they relate to drug responses, such as the examination of whole genomes in order to identify putative drug targets or to study large scale differences in gene expression in response to drugs.^{4,5}

Pharmacogenetic variation

The Human Genome Sequencing project has provided us with the sequence of the three billion base pairs that make up our DNA, of which is estimated that only 2% to 5% actually consists of the coding regions that are responsible for 25,000 – 32,000 genes. Background DNA variation is present throughout the whole genome, but tends to occur at a higher frequency in non-coding compared to coding regions. The most common variation consists of single base substitutions (single nucleotide polymorphisms, SNPs), of which nearly 1.8 million have been identified to date.⁶ Other types of DNA variation include deletions or insertions of one or more bases, and variable repeats of specific sequences. Pharmacogenetically-relevant DNA variation would be expected to be mainly located in coding sequences or in regulatory regions of genes which code for proteins involved in pharmacological responses. Such genes may include those responsible for drug receptors (e.g. β_2 -adrenoceptor, muscarinic receptors, glucocorticoid receptor) or proteins involved in drug receptor signalling (e.g. G_s) as well as genes which code for drug-metabolizing enzymes (e.g. cytochrome P450 group).

Pharmacogenetic variation relevant to asthma therapeutics

β_2 -adrenoceptors

β_2 -adrenoceptors are primarily expressed on airway smooth muscle cells⁷, and are the target of the β_2 -agonist drugs used in asthma. Nine SNPs have been identified in the

β_2 -adrenoceptor coding region, of which 4 result in amino acid substitutions at the protein level. Three of these have demonstrable functional effects.^{8,9}

A DNA base change of adenine to guanine at position 46 of the β_2 -adrenoceptor gene (46A→G), results in a receptor protein for which the sixteenth amino acid is glycine instead of arginine (Arg16→Gly). In cultured cells, the Gly16 receptor variant downregulates faster than Arg16 in the presence of agonists^{10,11}, while patient studies have shown homozygous Gly16 adult asthmatics to exhibit a greater degree of tolerance (described by a higher loss in positive FEV₁ or FEF₂₅₋₇₅ responses) to formoterol treatment (24µg b.d. for 4 weeks) compared to Arg16 homozygotes.¹² Arg16 adult homozygotes have been shown to demonstrate a higher and more rapid salbutamol-evoked FEV₁ response¹³ while Arg16 asthmatic children are 5.3 fold more likely to exhibit positive clinical responses to salbutamol treatment than Gly16 homozygotes.¹⁴ Gly16 asthmatic patients are 6 times more likely to suffer from nocturnal asthma symptoms¹⁵ and they demonstrate a higher degree of airway reactivity to histamine.¹⁶

A second polymorphism (Gln27→Glu, 76C→G) confers on the receptor a strong resistance towards agonist-promoted *desensitization* and *downregulation*. In primary cultures of human airway smooth muscle cells, approximately 60-fold greater concentrations of isoprenaline were required to desensitize the homozygous Glu27 variant to the same extent as the homozygous Gln27 form¹¹, while homozygous Glu27 patients have four-fold lower methacholine reactivity than their Gln27 counterparts.¹⁷

Work on Gly16/Glu27 double mutant receptors, showed the Gly16 effects to be dominant over Glu27 in cell culture.¹⁰ The highly downregulating Gly16/Gln27 variant has a higher prevalence in moderate than mild asthmatics¹⁸ and is associated with a higher degree of bronchial hyperresponsiveness.¹⁹

A third identified polymorphism (Thr164→Ile, 491C→T) is rare and population studies are lacking. *In vitro* work has identified the Ile164 variant

to bind isoprenaline, adrenaline and noradrenaline with 4-fold lower affinity than the wild type Thr164 form²⁰, and also to possess a reduced ability to mediate agonist-independent basal activation of adenylate cyclase, implying the existence of a second mechanism by which this variant transduces signal less efficiently.²¹

Various SNPs are present in the 1470bp DNA region upstream of the β_2 -adrenoceptor gene which is involved in transcriptional control of the gene. Cell culture studies have revealed that the most commonly occurring mutant haplotype (-20C, -47C, -367C, -468G) exerts a small but significant decrease in promoter activity compared to the wild type sequence²² and this may result in decreased β_2 -adrenoceptor expression in patients carrying this variant.

Muscarinic receptors

Polymorphic variation within muscarinic M₂ and M₃ receptors could potentially alter treatment responses to anticholinergic agents, such as ipratropium bromide. Mutation screening of the M₂ receptor gene in Maltese asthmatic individuals identified two degenerate polymorphisms in the coding region (1197T→C, Thr→Thr and 976A→C, Arg→Arg) and a common SNP in the 3' non-coding region (1696T→A), none of which are likely to be functionally relevant, while no variation could be identified in the M₃ coding sequence.²³ A third M₂ coding region degenerate polymorphism (1050A→G) was identified in the Japanese population while a rare degenerate M₃ substitution (261C→T) was identified in the M₃ coding region in the same population.²⁴ A recently identified variable CA tandem repeat in the human muscarinic M₂ gene promoter has been shown to significantly influence gene transcription in cultured cells²⁵ and ongoing work by our group strongly suggests that this variation may be contributory to the development of asthma symptoms in patients. It is suggested that these promoter variants may contribute to inter-individual variability in response to muscarinic antagonists (such as ipratropium bromide) due to their influence on muscarinic M₂ receptor expression.

Anti-leukotriene drugs

Cysteinyl leukotrienes are released into the airways by pro-inflammatory cells including eosinophils, neutrophils and mast cells, and bind to specific receptors (primarily CysLT₁) exerting effects which include airway smooth muscle contraction, plasma extravasation and mucus hypersecretion.²⁶⁻²⁸ The products are derived from arachidonic acid, via an enzymatic pathway in which 5-lipoxygenase (5-LOX) and leukotriene C₄ synthase (LTC₄S) exert primary roles.²⁹ Drugs which inhibit 5-LOX, (e.g. zileuton) or block receptors to which cysteinyl leukotrienes bind (e.g. zafirlukast, montelukast, pranlukast) are the latest addition to the available anti-asthma drugs, and they have a proven clinical efficacy in relieving symptoms.³⁰ The genes for the cysteinyl leukotriene receptors have only been recently cloned, and studies concerning genetic variation are currently underway.

The 5-LOX gene (ALOX5) is located on chromosome 10q11.12³¹, and the upstream flanking region has promoter activity and contains consensus sequences for several transcription factors, including Sp1, Sp3, Egr-1, Egr-2, NF-κB, GATA, Myb and AP family members³², including a series of 5 tandem binding motifs for Sp1/Egr-1 ([GGGCGG]₅).³³ Thirty five percent of the population carries an ALOX5 promoter with either one or two Sp1/Egr-1 sequences deleted ([GGGCGG]₄, [GGGCGG]₃) or the insertion of an extra one ([GGGCGG]₆). All 3 variants show decreased promoter activity in cell culture, compared to wild type [GGGCGG]₅. A study using ABT-761, a 5-LOX inhibitor derivative of zileuton, in 114 asthmatic patients, at a dose of 300mg/day for 84 days, showed the highest degree of improvement in FEV₁ to occur in patients who are heterozygous or homozygous for the wild type allele at the promoter locus, while patients who are homozygous mutant did not benefit from anti-5-LOX treatment.³³⁻³⁵

The gene for LTC₄ synthase (LTC₄S) is located on chromosome 5q35. Sanak, *et al.*, (1997) identified an A→C substitution in a regulatory region, 444 bases upstream of the coding sequence

which resulted in an additional motif for transcription factor AP-2 (CCCC). The polymorphism shows an association with aspirin induced asthma and could potentially contribute to increased LTC₄ in the airway.³⁶ It could also be a potential risk factor for adverse reactions to nonsteroidal analgesics in asthma, since it may alter the expression pattern of the enzyme.

Glucocorticoid receptor

Glucocorticoids (GCs) act by binding to a cytoplasmic receptor (GR), which subsequently enters the nucleus and through various mechanisms acts as a positive or negative transcriptional regulator. In this way, the transcription of various pro-inflammatory proteins is decreased, while there is transcriptional upregulation of anti-inflammatory molecules, such as lipocortins.^{37,38} Two isoforms of the human glucocorticoid receptor (hGR) exist, hGR α and hGR β , of which only hGR α can bind ligand. There is evidence to suggest that one role of hGR β is to dimerize with hGR α , creating a heterodimer that has less transcriptional regulatory activity than a normal hGR α homodimer³⁹, although some authors disagree on this.⁴⁰ Although the ligand-binding isoform is the better studied in the literature, polymorphic variation in either hGR α or hGR β may potentially exert an influence on glucocorticoid-mediated transcriptional regulation.

Notwithstanding the proven efficacy of GCs, there remain a subset of asthmatic patients who are GC-resistant.⁴¹ 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-1.⁴²

While various glucocorticoid receptor abnormalities have been reported to contribute to generalized inherited glucocorticoid resistance (GIGR), a rare disorder characterized by high cortisol levels with no Cushingoid features⁴³, studies identifying defined contributions of hGR variants to steroid resistance in asthma are currently lacking.⁴⁴ Examples of identified hGR variants include a Val641→Asp substitution which results in a three-fold lower binding affinity for

dexamethasone in COS-7 cells⁴⁵, a Val729→Ile substitution which results in a four fold decrease in dexamethasone activity⁴⁶ and a Asn363→Ser substitution which results 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.⁴⁷ A recent variant identified in leukaemic cells (Cys643→Arg) has been found to decrease steroid-binding affinity and transcriptional activity⁴⁸, while an Asn363→Ser variant has been correlated with increased glucocorticoid sensitivity, lowered bone mineral density and increased body mass index.⁴⁹ Although it may be expected that asthmatic patients carrying the Val641→Asp, Cys643→Arg or Val729→Ile GR variants may exhibit a decreased clinical response to glucocorticoid administration than the respective wild-type individuals, current evidence suggests that glucocorticoid resistance in asthmatics may be associated with variation in genes coding for other proteins involved in glucocorticoid-mediated pathways such as histone deacetylases.⁵⁰

Phosphodiesterase

At least 7 different phosphodiesterase enzyme families are expressed in humans, of which type 4 (PDE₄) represents the predominant cAMP hydrolyzing activity in human airway smooth muscle.⁵¹ Augmentation of PDE₄ activity might be expected to decrease β_2 -agonist response, by degrading β_2 -adrenoceptor mediated *de novo* 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*.^{52,53} 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.⁵⁴

Database searches suggest that phosphodiesterase genes contain a number of polymorphisms; however there are currently no available data on the mutation screening of phosphodiesterase genes in asthmatics.

Applications

One of the major aims of pharmacogenetic research is to develop DNA testing procedures that will predict how a particular patient will respond to a given drug, in terms of efficacy as well as adverse effects. On a clinical level, this will enable a more patient-focused prescribing, and will help to ensure that patients will receive the drugs that will benefit them most, at the dose which will provide the required clinical response. Pharmacogenetic tests may be used to stratify individuals participating in clinical trials, into pharmacogenetically homogeneous groups and this may lead to more robust scientific findings regarding the group of patients who might eventually be prescribed the medicine.⁵⁵ Pharmacogenetic knowledge may also help to develop drugs that will provide efficacy in a wider spectrum of patients, or promote the development of new drugs specifically designed for pharmacogenetically compromised patients.

Ethical considerations

The present status suggests that pharmacogenetic testing for specific drugs may be available sooner rather than later, and this oncoming is not devoid of ethical dilemmas. Pharmacogenetic testing may discourage pharmaceutical companies from developing medicines that would only provide benefit for a minority of patients. If pharmacogenetic testing is incorporated into the licensing conditions for specific drugs, this increased expense might adversely affect the cost-benefit equilibrium, thus potentially depriving patients who would particularly benefit from these drugs. A pharmacogenetic test might reveal more knowledge than is specifically intended. For example, a patient who is a rapid metabolizer for a particular drug, is likely to also rapidly metabolize other pharmacologically

unrelated drugs which share the same metabolic pathways. Should such additional information be disclosed to the patient? In the clinical setting, a patient might be expected to provide informed consent for a pharmacogenetic test to be carried out. The implications of such a test should be clearly explained, and the result should be accompanied by professional advice. Ethnicity may bear an influence on the validity of a pharmacogenetic test, since specific genotypes may only be present in particular populations. Will test developers take this into account, or will particular populations be sidelined due to marketing or financial considerations? Pharmacogenetic information may be requested by insurance companies, to aid in the computation of health insurance premiums, thus potentially dissuading patients from consenting to such tests for fear of having to pay higher premiums or being unable to obtain

insurance. It is applaudable that the UK has currently imposed a moratorium on the use of genetic and pharmacogenetic data for setting insurance premiums. This moratorium however expires in 2006.^{55,56}

Conclusion

Functional pharmacogenetic variation is often initially demonstrated using cell culture models. Although results obtained from such systems provide accurate descriptions of cell-based responses, this data cannot be automatically extrapolated to patients. Only after having studied genetic variants in clinical studies, can one obtain concrete evidence of the actual relevance to phenotype.

The discovery of a novel pharmacogenetic variant of high allelic frequency, may warrant modifications of standard treatment protocols in order to optimize management in a greater number of patients. On the other hand, identification of a rare pharmacogenetic

variant, which poses serious therapeutic implications, would allow for better management of selected patients who might otherwise be classified as difficult to treat. At present, the currently available data regarding asthma pharmacogenetics may not be sufficient to justify routine genotyping of all patients prior to treatment. However, as new data becomes available, and novel therapies are developed, the knowledge of patients' genotypes will be a necessary requisite in order to enable pharmaceutical companies and prescribers to optimize management of the disease. Further clinical and molecular work is needed in order to consolidate and expand current knowledge.⁵⁷⁻⁵⁹

The importance of this area of research has been accentuated by the recent UK Department of Health announcement of a commitment of £4 million over 3 years to be granted to pharmacogenetic research.⁶⁰

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