Heterogeneity of therapeutic responses in asthma

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Asthma is a complex clinical syndrome with multiple genetic and environmental factors contributing to its phenotypic expression. This aetiological heterogeneity adds to the complexity when addressing variation in the response to anti-asthma treatment. Currently, there are three main lines of treatment available: (i) inhaled glucocorticoids which have multiple mechanisms of action; (ii) β₂-agonists which are very effective bronchodilators and act predominantly on airway smooth muscle; and (iii) cysteinyl-leukotriene inhibitors. Analysis of the repeatability (r) of the treatment response, defined as the fraction of the total population variance which results from among-individual differences, shows values of r between 60-80% indicating that a substantial fraction of the variance of the treatment response could be genetic in nature. Among the sources of variability that could contribute to the observed heterogeneity in the response to treatment are the degree of underlying inflammation, such as in glucocorticoid resistance, and polymorphisms in the genes encoding the drug target, such as β₂-adrenoceptor and 5-lipoxygenase.

Bronchial asthma is a disease which is characterised clinically by episodic symptoms of wheeze, dyspnoea and chest tightness. Physiological abnormalities include a variable reduction in airflow which is secondary to increased airway resistance and bronchial hyper-responsiveness (BHR) to specific and non-specific agents such as allergen and histamine, respectively. Pathologically, even in the mildest of asthma cases, there is evidence for airway inflammation with a cellular infiltrate of mononuclear cells and eosinophils into the airway mucosa. These cells are present in increased numbers and are activated to secrete pro-inflammatory cytokines that may be pertinent to the pathophysiology of the asthmatic process.

Currently, there are three main asthma treatments available: (i) inhaled glucocorticoids; (ii) β₂-agonists; and (iii) leukotriene inhibitors. Bronchial asthma is a heterogeneous disorder, but even in patients with an apparently identical clinical phenotype, response to drug treatment may be remarkably variable. It is common for some patients to respond in...
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a salutary fashion to a given treatment while others fail to manifest such a response. The basis for this variable treatment response is not known with certainty, but there is good reason to believe that a significant component of the variability is genetic in nature. The basis for this statement derives from evaluation of the repeatability of the asthma treatment response. Repeatability is an upper limit estimate of the heritability of the treatment response ($h^2$) that can be made from data available from treatment trials of unrelated individuals\(^1,2\). The essence of this approach is to estimate the within-individual variance in the treatment response, assumed to be due to environmental variance and measurement error, and compared it with the total population variance in the treatment response, assumed to be due to genetic, environmental, and measurement errors\(^3\). Since the variance within a given subject of the most commonly used measurement of lung function, i.e. FEV\(_1\), is relatively small\(^4,5\) repeatability can be defined by the relationship $r = (V_C + V_{eg})/V_p$ where $V_C$ is the genetic variance, $V_{eg}$ is the general environmental variability which is defined\(^1\) as the ‘environmental variance contributing to the between-individual component and arising
from permanent or non-localised circumstances’ and $V_p$ is the total variance. The repeatability thus sets an upper limit to the degree of genetic determination. We used the data available from asthma treatment trials to estimate ‘$r$’ in the treatment response to inhaled $\beta_2$-agonists, to inhaled steroids, and to leukotriene modifiers (Fig. 1).

We estimated the percentage of variation in the FEV$_1$ response to salbutamol, that was attributable to between individual differences, which corresponds to the maximal possible percentage attributable to genetic causes, i.e. ‘$r$’; it constituted 60.6% of the total variance. We estimated the among-patient variability and within-population variability from the final percent of the predicted FEV$_1$ achieved after 6 weeks of treatment with inhaled triamcinolone acetonide and found a value of ‘$r$’ of 86.1%, thus providing evidence that a clinically significant component of the treatment response to inhaled corticosteroids is likely heritable. Finally, we estimated ‘$r$’ from a data set obtained in a clinical trial of ABT-761, a 5-lipoxygenase (ALOX5) inhibitor, in which 110 patients were treated with high-dose ABT-761 for 12 weeks. The within-patient variability and within-population variability were estimated as noted above for $\beta_2$-agonists, leading to a value of ‘$r$’ of 61.2%. Thus, our findings indicate the potential for a substantial component of the treatment response being inheritable in nature. Our analysis of existing treatment trials indicates that at least half of the observed variance of the treatment response to inhaled $\beta_2$-agonists, to inhaled steroids, and to leukotriene modifiers can be ascribed to among-patient differences. This value represents an upper limit of the genetic variability and indicates that a clinically significant fraction of the total population variance is potentially genetic in origin. With this as a background it makes sense to examine evidence for association between the drug treatment response and known genetic variation in treatment outcomes.

**Glucocorticoid therapy – variable treatment responses**

Although glucocorticoids (GCs) are the mainstay of treatment for bronchial asthma, there has been increasing recognition of a group of asthmatic patients who do not appear to benefit from glucocorticoid therapy, i.e. the GC-resistant (GCR) asthmatic. GC responsiveness is probably a continuous spectrum with individuals who demonstrate GC resistance falling at one end of a unimodal distribution. For clinical purposes, a common definition of GC resistance is the failure of an asthmatic patient to improve FEV$_1$ by 15% from a baseline of ≤75% predicted after an adequate dose (e.g. ≥40 mg prednisolone) for an adequate duration of time (e.g. 1–2 weeks), despite demonstrating greater than 15% reversibility to an inhaled $\beta_2$-agonist and provided compliance was ensured.
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This is a pragmatic definition, which nevertheless is useful because it defines a sufficiently high dose of glucocorticoid and a duration of usage after which physicians would feel uncomfortable in maintaining patients on continuous systemic GCs. GCR asthma was first described by Schwartz and colleagues in 1967. In 1981, Carmichael described 58 subjects with chronic asthma who were clinically resistant to prednisolone therapy. Compared with GCS subjects, these patients had a longer duration of asthma, a more frequent family history of asthma, poorer morning lung function, and a greater degree of bronchial reactivity. These early clinical studies suggested that both genetic (family history of asthma) and environmental (longer duration of asthma) factors may play a role in the pathogenesis of this condition.

Cellular abnormalities in glucocorticoid resistant asthma

In view of the lack of evidence for any gross biochemical abnormality in these patients, subsequent studies focused on the role played by peripheral blood mononuclear cells in GCR asthma. Many studies have now demonstrated that GCR asthma is associated with impaired in vitro and in vivo responsiveness of monocytes and T lymphocytes to the suppressive effects of GCs.

Corrigan has shown enhanced interleukin-2 (IL-2) and HLA-DR receptor expression on peripheral T lymphocytes in GCR as opposed to GCS asthma. In addition, he has shown that PHA-induced T cell proliferation and the elaboration of IFN-γ and IL-2 from mitogen-stimulated T lymphocytes was inhibited by dexamethasone in GCS, but not in GCR subjects. Interestingly, cyclosporin-A was seen to partially reverse this in vitro resistance, suggesting a potentially therapeutic role for this treatment in GCR asthma.

Leung has examined the effects of a 1 week course of prednisolone on BAL cells obtained from patients with GCR asthma. It was shown that GCR subjects had elevated cell numbers expressing IL-2 and IL-4 before prednisolone treatment as compared to the GCS subjects. In contrast to GCS subjects, prednisolone failed to suppress IL-4 and IL-5 expression in the GCR subjects. Therefore, the airway cells from patients with GCR compared with GCS asthma have different patterns of cytokine gene expression and distinct responses to GC therapy.

Tissue specificity of glucocorticoid resistance in asthma

We have examined whether the lack of clinical response to GCs seen in GCR bronchial asthma is reflected in abnormalities of endogenous
cortisol secretion and in the sensitivity of the hypothalamic-pituitary-adrenal (HPA) in GCR subjects by using a modification of the standard dexamethasone suppression test in response to 0.25 mg and 1 mg oral dexamethasone\(^{13}\). The data indicate that GCR asthma is not reflected in an altered secretory rate of endogenous cortisol or in a different sensitivity of the HPA axis to dexamethasone suppression. In order to assess whether GCR asthmatic patients are equally at risk from the side effects of GCs on bone metabolism, GCS and GCR asthmatic patients received prednisolone 40 mg orally for 5 days. Prednisolone suppressed osteocalcin equally in both the GCS and GCR groups\(^{14}\). These two studies indicate that ‘non-immune’ tissue responds normally to GCs in GCR asthma and that these subjects are, therefore, equally at risk of Cushingoid side effects. An explanation of these findings is that the presence of inflammation in these cells is a necessary prerequisite for the unmasking of the resistant profile.

**Glucocorticoid bioavailability in glucocorticoid resistant asthma**

Interest has also focused on whether impaired bioavailability of GCs can account for the differences in therapeutic responses in steroid dependent and steroid resistant asthma. May et al measured the pharmacokinetic profile of a single dose of 15 mg oral prednisolone in 12 steroid-dependent asthmatic subjects by radioimmunoassay (RIA) and found no inter-individual differences in these subjects with respect to \(C_{\text{max}}\), plasma half life and area under the concentration/time curve: they concluded that differences in prednisolone bioavailability is not a factor in determining the dose required to control asthma\(^{15}\). Rose and colleagues observed that the plasma protein binding, distribution and clearance of prednisolone are not responsible for the large prednisolone requirement of steroid-dependent asthmatics. He extended the above studies to GC dependent and resistant asthmatic children and again found no difference in bioavailability parameters. We have examined the pharmacokinetic profile of an oral dose of 40 mg prednisolone in GCS and GCR asthmatic subjects\(^{16}\). We found that there was no significant difference in AUC, \(C_{\text{max}}\) and estimated clearance values between the normal group studied and each of the asthmatic groups. This implies that clinical GC resistance in asthmatic subjects is not reflected in any gross abnormality of the absorption or elimination of prednisolone. These data are in agreement with pharmacokinetic studies carried out in GCR asthma by other groups who observed no differences in estimated clearance values of a single dose of oral prednisolone between groups of well characterised GCR and GCS asthmatic subjects\(^{11}\).
Glucocorticoid receptor characteristics in glucocorticoid resistant asthma

The mechanisms of glucocorticoid action are summarised in Figures 2–4. Competitive binding studies on nuclear extracts derived from peripheral blood monocytes using \(^{3}\)H-dexamethasone have demonstrated no difference in the \(K_d\), \(R_o\) or nuclear translocation of the activated receptor complex between GCS and GCR asthmatics\(^{17}\). We have shown that GC resistance in bronchial asthma cannot be explained by abnormalities in receptor nuclear translocation, density or binding affinity. It is possible that the phenomenon of GC resistance may be heterogeneous. Indeed, a 4-fold reduction in receptor binding affinity has been described in T-cells. However, the authors concluded that such a small reduction in \(K_d\) was insufficient to explain the gross difference in GC responsiveness at the clinical level\(^{11}\). Similarly, Sher et al have described two patterns of ligand

![Diagram of glucocorticoid action](image)

**Fig. 2** Glucocorticoids mediate their effects via the glucocorticoid receptor (GR) which is present in the cytoplasm of all cells. Glucocorticoids enter the cell by passive diffusion where they bind to the GR non-covalently by hydrophobic and hydrogen ion interactions. This results in a conformational change in the GR described as activation. This process modifies the receptor allosterically, whereupon the GR undergoes dephosphorylation, dissociates into two 90 kDa associated heat shock proteins, forms dimers and translocates into the nucleus where it binds to sequences in DNA known as glucocorticoid response elements (GREs) in the promoter regions of the glucocorticoid responsive genes, leading to either induction of inflammatory genes or suppression of anti-inflammatory genes.
binding abnormalities in their group of GCR asthmatics termed type 1 and 2\(^1\). The more common type 1 defect was associated with ‘Cushingoid side effects’, reduced \(K_d\), normal receptor numbers, localisation to T-cells, reversibility with serum deprivation and was IL-2 and IL-4-dependent. The less common type 2 defect was associated with reduced receptor density with a normal \(K_d\), was irreversible and was seen in the total mononuclear cell population. The type 1 defect is acquired as a result of long-standing inflammation whereas the type 2 defect is more likely to be a genetic defect.

We have demonstrated a reduction in the binding of the activated glucocorticoid receptor complex to its GRE using gel retardation assays in nuclear extracts from mononuclear cells obtained from GCS, GCR and non-asthmatic control subjects\(^1\). Dexamethasone was seen to induce a significant rapid and sustained 2-fold increase in GRE binding in the mononuclear cells from the GCS subjects and non-asthmatic

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**Fig. 3** Inducible AP-1 is formed by dimerisation of c-Fos and c-Jun following dephosphorylation of Jun N-terminal kinase (JNK). AP-1 binds to the TPA response element (TRE) to activate genes encoding for inflammatory cytokines. Glucocorticoid receptor inhibits AP-1 either by direct sequestration or by spatial interference. +ve indicates activation and −ve indicates inhibition.
Fig. 4 Activation of NF-κB by, for example, cytokines can be blocked by glucocorticoids. Activation of NF-κB involves phosphorylation and subsequent proteolytic degradation of the inhibitory protein IκB by specific IκB kinases. The free NF-κB (p50 and p65 heterodimer) can then translocate to the nucleus where it binds to κB sites in the promoter genes of inflammatory proteins. Glucocorticoid receptor (GR) complexes bind to the p65 subunit of NF-κB preventing NF-κB activation of inflammatory genes. Synthesis of IκBα is promoted by the binding of glucocorticoid–GR complexes to a glucocorticoid responsive element in the promoter region of the IκBα gene.

control subjects that was significantly ($P < 0.005$) reduced in the GCR subjects. These data have recently been confirmed by other investigators who have suggested that this defect may occur as a result of an IL-2- and IL-4-dependent increase in the pro-inflammatory isoform of the glucocorticoid receptor\textsuperscript{20}. These data suggested that there may be a mutation(s) in the primary structure of the glucocorticoid receptor, particularly in its DNA-binding domain which could account for this functional abnormality.

We have tested whether the reduction in DNA binding of the glucocorticoid receptor is due to polymorphisms in its primary structure using the sensitive technique of chemical mutational analysis (CMA)\textsuperscript{21}. Using this technique we did not detect any base pair mismatch between the 6 GCS and 6 GCR patients and the corresponding wild type glucocorticoid receptor, despite a 100% detection of control mutations indicating that in these patients the defect in GCR asthma does not lie in the structure of the
glucocorticoid receptor. This was further confirmed by dideoxy sequencing using linear PCR elongation and chain termination. Therefore, a defect in the primary structure of the glucocorticoid receptor does not account for the reduction of in vitro DNA binding and prompted us to further examine the binding characteristics of receptor-DNA interaction. Subsequent Scatchard analysis indicated that the defect in the GR subjects was underpinned by a reduction in the numbers of nuclear translocated glucocorticoid receptors available for DNA interaction. This was despite there being an equal amount of nuclear translocated receptors available on the basis of previous ligand binding experiments. These data indicated to us that the DNA binding sites of the glucocorticoid receptors were being competed out, possibly by pro-inflammatory transcription factors. These factors are bound by glucocorticoid receptor DNA binding sites and so prevent their subsequently activating pro-inflammatory cytokine genes. Thus the reduction in the ‘read-out’ of DNA binding of the GRE may reflect the presence of excess pro-inflammatory activity rather than a direct problem with gene transcription per se. It is, therefore, important to review the mechanisms of negative gene regulation by GCs.

Transcription factor interactions in glucocorticoid resistant asthma

We have examined whether the functional abnormality of reduced DNA binding of the glucocorticoid receptor in GCR asthma is caused by increased activity of pro-inflammatory transcription factors. We examined the activities of AP-1, NF-κB and CREB using gel shift assays in nuclear extracts from unstimulated PBMCs obtained from GCR and GCS subjects and found that AP-1, but not NF-κB and CREB, DNA binding was significantly (P <0.01) increased approximately 2-fold in the GCR subjects. In order to further understand these mechanisms, we sequenced the c-jun and c-fos major components of AP-1 and found no evidence for polymorphism in their primary structure. We have recently shown that T lymphocytes and monocytes from GCR subjects generate a 2-fold excess of Fos protein which is secondary to an increase in the c-fos transcription rate. We were able to suppress glucocorticoid receptor DNA binding in GCS subjects to levels seen in GCR subjects by a PMA inducible factor, which we have shown by co-immunoprecipitation studies to be c-Fos. Therefore, GCR subjects generate excess c-Fos which results excess AP-1 activity. This would result in perpetuation of AP-1-mediated inflammation and would render the therapeutic effects of GCs less effective by sequestration of glucocorticoid receptors within the nucleus.

We then used the tuberculin-induced model of dermal inflammation to evaluate the effect of corticosteroids in regulating components of AP-1 in vivo on 9 GCS and 6 GCR asthmatic subjects for the regulatory
components of AP-1 before and after 9 days of either of 40 mg prednisolone or placebo. Significantly greater expression of c-Fos, phosphorylated c-Jun and phosphorylated Jun amino terminal kinase (JNK) protein has been identified in GCR compared to GCS subjects. Corticosteroids suppressed phosphorylation of c-Jun and JNK in the GCS group, but enhanced phosphorylation of c-Jun and JNK in the GCR group\(^26\).

Although we have yet to define a genetic mechanism, it is clearly established that there is excess AP-1 activity in GCR asthma. This molecular observation is consistent with the clinical observation that prolonged untreated asthma renders the subsequent response to GCs less effective. We speculate that this occurs as a result of chronic unopposed AP-1-mediated inflammation. If true, then early suppression of inflammation by GCs would predict a more favourable outcome in asthma by early suppression of AP-1-mediated inflammation.

\section*{$\beta_2$-Agonist therapy}

$\beta_2$-Adrenoceptor agonists are used by virtually all patients as rescue bronchodilator\(^{27,28}\). Short and long-acting $\beta_2$-agonists exhibit protective effects against a variety of direct and indirect bronchoconstrictor stimuli. However, regularly scheduled treatment with $\beta_2$-agonists is associated with tachyphylaxis to the functional antagonism against bronchoconstrictor stimuli. There is evidence to show that inhaled corticosteroids and long-acting $\beta_2$-agonists given on a regular basis have additive effects in improving long-term asthma control. The debate about the safety of regular $\beta$-agonist use in asthma has evolved substantially over the past decade. In spite of epidemiological data suggesting an association between $\beta$-agonist use and asthma deaths and an early study suggesting decreased asthma control on regular fenoterol\(^{29}\), the overwhelming message from subsequent, large clinical studies, has been reassuring\(^{30,48}\).

\section*{$\beta_2$-Adrenoceptor polymorphism}

One of the remaining issues in this debate is whether there might be a subgroup in the asthmatic population which does not benefit from regular $\beta_2$-agonist use, or which may be more vulnerable to rapid deterioration. One potential source of such an anomalous response could be DNA sequence variants within the $\beta_2$-adrenoceptor ($\beta_2$AR) gene. This intronless gene was cloned in 1987 and is situated on the long arm of chromosome 5 (5q 31-33). Nine polymorphisms have been described within the single coding region. Although five are degenerate, the four remaining polymorphisms result in single amino acid substitutions: glycine for arginine at
amino acid 16, glutamate for glutamine at amino acid 27, methionine for valine at 34 and isoleucine for threonine at position 164. Amino acids 16 and 27 lie in the extracellular N-terminal domain, whereas amino acids 34 and 164 are in the transmembrane spanning regions. These polymorphisms have been found with equal frequency in normal and asthmatic populations\textsuperscript{31} and are unlikely to be a cause of asthma \textit{per se}, although they may influence the phenotype of the illness once it is expressed.

\textit{In vitro} studies have shown that these polymorphisms have potential functional consequences. The relatively uncommon Ile-164 polymorphism results in a major decrease in agonist binding affinity and coupling to adenylate cyclase. Polymorphisms Gly-16 and Glu-27 did not affect receptor binding or coupling but markedly altered agonist-promoted receptor downregulation and functional desensitisation; Gly-16 enhanced this down-regulation, whereas Glu-27 protected against it.

There are a number of recent clinical studies relating to the Gly-16 and Glu-27 polymorphisms, because the frequency of the minor allele is on the order of 40–50%; as would be expected there is strong linkage disequilibrium between these two sequence variants making it difficult to assign an effect to a single allele in association studies. A relatively early report suggested that homozygous Gly-16 was associated with a more severe asthma phenotype\textsuperscript{31}, but this has not been supported by more recent studies\textsuperscript{32}. Gly-16 has also been associated with nocturnal asthma\textsuperscript{33} and in children it has been reported to be associated with decreased bronchodilator response to an inhaled $\beta_2$-agonist\textsuperscript{34}. The potential protective Glu-27 polymorphism has indeed been reported to be associated with decreased airway reactivity in asthma\textsuperscript{35} but it did not seem to influence nocturnal asthma\textsuperscript{33} or bronchodilator responsiveness\textsuperscript{34}. The Gln-27 allele, on the other hand, has been associated with elevated IgE levels and with an increase in self-reported asthma in children.

Lipworth \textit{et al}\textsuperscript{36} have recently reported that Gly-16 increased the propensity for bronchodilator desensitisation after the regular use of formoterol, although whether such tachyphylaxis actually occurs has been quite controversial, with most groups failing to find any drop-off in the bronchodilator effectiveness of $\beta$-agonists when they are used long-term in populations without genotype stratification. Lipworth \textit{et al} pursued tachyphylaxis to bronchoprotection further and investigated the relationship of the common $\beta_2$AR polymorphisms to the fall-off in protection afforded by $\beta_2$-agonists against induced bronchoconstriction when in regular use (bronchoprotective subsensitivity or tachyphylaxis). $\beta_2$-agonist subsensitivity occurred irrespective of variations in $\beta_1$AR genotype. They found no association with the Glu-27 polymorphism although this may have been confounded by its linkage disequilibrium with Gly-16. At lower doses of formoterol, whereas the Gly-16 homozygotes showed uniform marked loss of protection, there was a much greater variability when Arg-16 allele was present.
Recently, Hancox and colleagues reported the genotypes of the patients originally reported in 1990, where they studied the effects of regular versus as-needed fenoterol in a group of 64 asthmatics. The earlier report showed a deterioration in 'asthma control' with regularly scheduled fenoterol treatment compared with as-needed. These investigators have now assessed \( \beta_{2}AR \) genotype at positions 16 and 27 in 61 of these subjects to determine whether changes in these variables in the earlier study were associated with \( \beta_{2}AR \) polymorphisms. During treatment with regularly scheduled fenoterol, subjects who were homozygous for Arg-16 had an increase in bronchial responsiveness to methacholine compared with their responsiveness when they did not receive regular treatment. This finding is consistent with patients who are homozygous for the Arg 16 form of the \( \beta_{2}-AR \) being predisposed to an adverse reaction to the regularly scheduled use of this \( \beta \)-agonist.

These findings have been corroborated by results obtained from the United States National Institutes of Health sponsored Asthma Clinical Research Network. They had previously shown, in a 16-week, randomised, placebo-controlled, double-blind, crossover trial in 255 patients with asthma, that, on average, regularly scheduled (2 puffs 4 times/day) salbutamol had no clinical or physiological deleterious effects compared with the use of salbutamol on an as-needed use schedule. Although deleterious effects were not observed in the entire group, some individuals did display tachyphylaxis, particularly in the group allocated to regular salbutamol use. Genotyping at the 16th and 27th amino acids of the \( \beta_{2}AR \) was carried out with genomic DNA obtained from the patients after additional informed consent in 190 patients. Individuals with the Arg/Arg genotype receiving regularly scheduled salbutamol experienced a significant decline in morning PEFR over the course of the study \((P = 0.012)\). This tachyphylaxis was not observed when treatment was given on an as-needed basis to patients with the same genotype. In contrast, those with the Gly/Gly genotype, even when receiving regularly scheduled albuterol, showed no tachyphylaxis. A similar pattern was observed with evening PEFR. The findings were not influenced by the position 27 genotype, race, sex, or initial lung function. These data are consistent with polymorphisms of the \( \beta_{2}AR \) at locus 16 being a significant genetic predictor of tachyphylaxis to the \( \beta \)-agonist salbutamol. Indeed, these results are consistent with the notion that the Gly-16 receptor is down-regulated maximally by endogenous catecholamines and thus cannot display additional down-regulation by salbutamol. On the other hand, the Arg-16 receptor is less down-regulated by endogenous catecholamines, but is down-regulated with chronic administration of salbutamol. This concept of dynamic regulation is consistent with the results of Martinez et al., who showed a decreased initial response to salbutamol in patients homozygous for Gly-16.
Taken together, these data clearly indicate that genotype at the $\beta_2$AR can influence the response to a variety of treatment regimes with agents acting at this receptor. However, at present no clear picture has emerged about the relative importance of genotype on treatment outcomes and further study will be required before treatment recommendations stratified by genotype can be made.

**Anti-leukotriene therapy**

The newly released family of specifically targeted asthma treatments, namely agents that interfere with the synthesis or action of the leukotrienes, provide a previously unavailable method to identify a subset of patients in whom leukotrienes are key contributors to the expression of the asthma phenotype. These agents are produced when the 5-lipoxygenase (ALOX5) pathway, the name given to the series of biochemical reactions which result in the transformation of arachidonic acid, which is esterified in membrane phospholipids into leukotrienes, is activated.

There are a number of observations suggesting that leukotrienes contribute to the pathogenesis of asthma. Firstly, they can induce many of the abnormalities seen in asthma, including airway obstruction, bronchovascular leak, mucus gland secretion, and granulocyte chemotaxis. Secondly, they are, on a molar basis, among the most potent effector molecules known to cause airway obstruction. Thirdly, leukotriene $E_4$ (LTE$_4$), an end-product of leukotriene metabolism, can be recovered in increased amounts from the urine of individuals with allergic asthma after antigen challenge. In addition, elevated levels of urinary LTE$_4$ are present in over two-thirds of patients presenting for the emergency treatment of asthmatic airway obstruction.

Leukotriene action may be pharmacologically modulated by antagonism at the receptor site or by biosynthesis inhibition. Both LTE$_4$ receptor antagonism and inhibition of ALOX5 biosynthesis have been shown to be effective anti-asthma treatments. There are several antileukotriene drugs which have been approved for the treatment of asthma. Zileuton is an inhibitor of ALOX5, while zafirlukast, pranlukast and montelukast are inhibitors of the action of LTD$_4$ at its receptor. A large number of clinical trials have been completed with these agents, establishing their efficacy in the treatment of spontaneous or induced asthma. All drugs have been shown to provide a superior effect, when compared to placebo, in the treatment of patients with mild-to-moderate asthma.

**5-Lipoxygenase gene promoter polymorphisms**

The failure of a patient with asthma to respond to antileukotriene treatment provides evidence consistent with the hypothesis that leukotrienes are
not critical to the expression of the asthmatic phenotype in that patient. Among the pharmacogenetic causes of this clinical phenotype are genetic variants that down-regulate gene expression of ALOX5. A family of polymorphisms exists in the core promoter of the ALOX5 gene. They consist of an alteration in the number of tandem Sp-1 and Egr-1 (early growth response protein) consensus binding sites, from the deletion of one or two or addition of one zinc finger binding sites in the region 176 to 147 base pairs up-stream from the ATG translation start site. The wild-type contains five such tandem repeats. 5-Lipoxygenase promoter reporter constructs containing these polymorphisms display less capacity for promoter binding and direct less gene transcription than constructs containing the wild-type ALOX5 core promoter. It seemed possible that such polymorphisms could explain a fraction of the heterogeneous therapeutic response to ALOX5 inhibition in asthmatic patients.

We reasoned that a patient harbouring any of the mutant forms of the ALOX5 core promoter would have decreased ALOX5 gene transcription and thus down-regulated ALOX5 expression. If ALOX5 products were among the several mediators contributing to the airway obstruction in asthma, and if patients harbouring the mutant genotypes had decreased leukotriene production, then these patients would have airway obstruction mediated by mechanisms other than the leukotrienes. As a consequence of this 'natural inhibition', patients harbouring the mutant genotypes would have a diminished response to exogenous ALOX5 inhibition. To test this idea, we examined data from a 12 week randomised, placebo-controlled and double-blind clinical trial of the effect of an ALOX5 inhibitor, ABT-761, stratified by genotype at the ALOX5 core promoter locus.

Patients with moderate asthma were treated with ABT-761 (high or low dose) or placebo for 12 weeks during which measurements of the FEV\textsubscript{1} were made at intervals; the FEV\textsubscript{1} was the primary outcome indicator. A total of 325 patients completed the entire protocol of whom about one-third received high dose ABT-761 therapy; this yielded enough patients to demonstrate superior efficacy of ABT-761 compared with placebo when FEV\textsubscript{1} was used as the outcome indicator. As expected from our previously published work, an allele frequency of 0.81 was found. Among the mutant alleles, most were deletion alleles S, that is alleles at this locus with fewer than 5 tandem repeats of the Sp-1 binding domain; the allele frequencies for alleles with 3 and 4 tandem Sp-1 binding motifs were 0.038 and 0.172, respectively.

Among the individuals receiving active treatment, patients with two wild-type alleles had a significantly greater FEV\textsubscript{1} at the completion of the trial than patients with no wild-type alleles (Fig. 5). The average change in the FEV\textsubscript{1} at the end of the active-treatment period was 18.8 ± 3.6% in wild-type patients and −1.1 ± 2.9% in mutant patients; the degree of change was significantly (P = 2.5 × 10\textsuperscript{-5}) greater in the former group than
in the latter. Although there were patients in whom a salutary therapeutic response was not observed who harboured the wild-type genotype, patients without wild-type alleles failed to manifest a salutary therapeutic response. This suggests that there are multiple ways for a patient to manifest a failed therapeutic response, of which a mutation in this core promoter genotype is one. It seems likely that a panel of DNA sequence variants at various loci in the ALOX5 pathway will be needed to explain all the observed variance in this novel form of asthma treatment.

**Summary**

Our understanding of asthma and its therapy has changed markedly over the last few years, particularly with the application of molecular and cell biology and the discovery of new and more specific pharmacological tools. It is timely to use these novel technologies to elucidate the mechanism(s) of heterogeneity of responsiveness to drug therapy, as this may provide insight into optimal targeting of drug therapy to subgroups of asthmatic patients. This may, in turn, provide greater understanding of underlying pathogenetic mechanisms.
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