

REVIEW

Pharmacogenetics of β_2 adrenergic receptor gene polymorphisms, long-acting β -agonists and asthma

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Summary

Adrenergic β_2 receptor (*ADR β_2*) agonists are widely used in asthma. Approximately 10% of patients have severe, poorly controlled disease despite extensive use of *ADR β_2* agonists. Variations in responses to *ADR β_2* agonists can, in part, be attributed to genetic variation, with 49 different polymorphisms having been identified for the *ADR β_2* gene. Although clear associations exist between *ADR β_2* gene polymorphisms, such as +46G > A, and patient response, the importance of these polymorphisms remains controversial. Patient selection, the number of polymorphisms analysed, differences in the type/dose of *ADR β_2* agonist, use of inhaled corticosteroids and population sizes have all varied. Most studies were limited to mild or moderate asthmatics using *ADR β_2* agonists sparingly. It is difficult to extrapolate from these studies to individual patients who have severe asthma, use a variety of *ADR β_2* agonists and do so frequently. The extent to which *ADR β_2* gene polymorphisms are relevant to asthma management needs further review, both clinically and at the molecular level. *In vitro* studies have helped to define the functional changes induced by specific *ADR β_2* gene polymorphisms, including 3'-untranslated region poly-C repeat. The resulting *ADR β_2* gene haplotypes (rather than genotypes), the interactions among *ADR β_2* gene haplotypes and variations in the chemistry of different agonists deserve more detailed assessment. Responses to *ADR β_2* agonists depend on effective downstream signalling following *ADR β_2* activation and also on receptor regulation. Studies on other regulators of *ADR β_2* receptor signalling and trafficking may be equally important in understanding the functional role of *ADR β_2* gene polymorphisms. The role of *ADR β_2* gene polymorphisms in the pathogenesis and management of severe asthma cannot be clearly defined until more specific and targeted research studies are performed.

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Introduction

Asthma is a complex, chronic disease that results in significant morbidity and mortality. While the vast majority of patients can be effectively treated using conventional medical therapy, a subgroup remains troubled by poorly controlled, severe persistent disease, leading to frequent hospital admissions, emergency department attendances, and symptoms that affect their quality of life.

Significant variation in response to medications has been observed in this group of patients. The reasons for this are multiple and complex. Although adherence to treatment is a major issue, it is estimated that up to 70% of the variability in therapeutic responses to pharmacotherapy is genetically determined [1–3]. Pharmacotherapy

that is tailored to an asthmatic patient's genotype should result in a clinically significant increase in efficacy and a reduction in adverse events.

β -Agonists are the most commonly used agents for the treatment of asthma. Patients with mild intermittent asthma are often managed with short-acting β -agonists (SABA), which are used as required. Current guidelines for treatment recommend a stepwise approach, with the addition of inhaled corticosteroids (ICS) and long-acting β -agonists (LABA) to improve symptom control as the disease severity increases [4]. Addition of a LABA to an ICS improves symptoms and reduces exacerbations in patients with persistent asthma [5–8], and is the preferred treatment approach compared with the use of high-dose ICS alone [4].

Despite the central therapeutic role of adrenergic β_2 receptor ($ADR\beta_2$) agonists, there are data that paradoxically suggest that inhaled $ADR\beta_2$ agonists may worsen clinical outcomes and possibly increase mortality in certain patient populations. In New Zealand, the use of both isoprenaline (a potent non-specific SABA) and fenoterol was associated with increased asthma mortality, which declined when the prescription of these drugs was stopped [9–11]. Increased prescribing of SABA has also been associated with higher asthma mortality [5, 12, 13], but the basis for the association is unclear. It could be a pharmacogenetic-related adverse effect, or equally, it may reflect poor asthma control, which independently increased the risk of death due to asthma. Significant increases in asthma and respiratory-related mortality were reported in two trials, in which patients were randomized to LABA monotherapy [14, 15]. Castle and colleagues [14] reported a statistically non-significant but alarming three-fold increase in the risk of asthma mortality with salmeterol administered twice daily compared with salbutamol administered four times daily. In the Salmeterol Multi-center Asthma Research Trial, there was an increase in mortality, but only among the African American patients enrolled in the study [15]. While this might reflect genotypic variation in the $ADR\beta_2$ clearly, this could reflect the lack of inhaled steroid use in these patients and also differences in non-pharmacological factors, such as genetic variation or socio-economic and psychosocial issues that contribute to poor disease control.

Polymorphisms within the promoter, coding and 5'- and 3'-untranslated regions (UTR) of the $ADR\beta_2$ gene, have been identified and shown to result in altered receptor expression, function and regulation. Such genetically based differences may account for some of the variability in responses to $ADR\beta_2$ agonists, and may potentially contribute to increased mortality in selected patient populations. Data on the functionality of $ADR\beta_2$ polymorphisms are limited to genotypes rather than haplotypes. $ADR\beta_2$ haplotypes should be more predictive of *in vitro* and *in vivo* responses to $ADR\beta_2$ agonists but the studies performed so far have been inconclusive. Cloning of common $ADR\beta_2$ haplotype constructs including the 3'-UTR polycytosine (poly-C) repeat into COS-7 cells demonstrated differences in receptor expression and agonist-promoted down-regulation between haplotypes [16]. More importantly, two haplotypes showed high $ADR\beta_2$ expression and less down-regulation, which may translate to better initial bronchodilator response with intermittent use of $ADR\beta_2$ agonist and less tachyphylaxis when treated with chronic therapy. However, current *in vivo* haplotype studies have not incorporated the functional effects of novel polymorphisms in the 3'-UTR poly-C repeat, which may prove to be crucial.

Some studies have clearly shown that $ADR\beta_2$ polymorphisms are associated with differences in clinical

responses to $ADR\beta_2$ agonist therapy [17–27]. Although this remains controversial, the significance of $ADR\beta_2$ polymorphisms in patients with severe asthma has not yet been adequately addressed. It is possible that the importance of $ADR\beta_2$ polymorphisms only becomes apparent when individuals with severe asthma use $ADR\beta_2$ agonists in excess.

Pharmacogenetic studies of associations between $ADR\beta_2$ polymorphisms and asthma vary considerably with respect to patient selection, study design and the use of therapy. Commonly used LABA such as formoterol and salmeterol differ significantly in their chemical properties. In some studies, down-regulation of $ADR\beta_2$ and tachyphylaxis were greater with formoterol than with salmeterol. This requires further confirmation *in vitro* and *in vivo* in patients in whom the $ADR\beta_2$ haplotypes are known. It is equally unclear whether differences in receptor expression and down-regulation related to $ADR\beta_2$ polymorphisms are modified by chronic treatment with corticosteroids. Functional changes resulting from $ADR\beta_2$ polymorphisms may be modulated by other regulatory elements of the $ADR\beta_2$ signalling and/or trafficking pathway, or play a role in their own right, and this represents fertile ground for future research.

This review article summarizes the significance of $ADR\beta_2$ polymorphisms and haplotypes in the management of asthma not only with SABA but also LABA therapy, and also highlights areas that require further evaluation.

Adrenergic β_2 receptor activation and desensitization

The $ADR\beta_2$ adrenergic receptor is a member of the seven transmembrane, G-protein-coupled receptor family. According to conventional dogma, activation of the $ADR\beta_2$ receptor leads to an increased activity of adenylyl cyclase, an enzyme that catalyses the conversion of ATP to cAMP [28] (Fig. 1). cAMP binds to the regulatory unit of protein kinase A, promoting the release of its catalytic unit, which phosphorylates a number of downstream target proteins. cAMP inhibits calcium release from intracellular stores and decreases calcium influx across the membrane, leading to bronchial relaxation and airway dilatation. It has been suggested that $ADR\beta_2$ agonist-induced bronchial relaxation may also occur through a cAMP-independent pathway, involving the direct interaction of stimulatory G protein with potassium channels in airway smooth muscle cells [28–31]. $ADR\beta_2$ activation also enhances the anti-inflammatory activity of corticosteroids by increasing the translocation of the glucocorticoid receptor (GR) from the cytoplasm to the nucleus of the cell [32]. This constitutes one of the many molecular mechanisms underlying the synergistic effects of $ADR\beta_2$ and GR activation.

Activation of the receptor by agonist also initiates negative feedback through receptor desensitization [29] (Fig. 1). The regulatory mechanisms controlling $ADR\beta_2$ desensitization and down-regulation after agonist binding are complex,

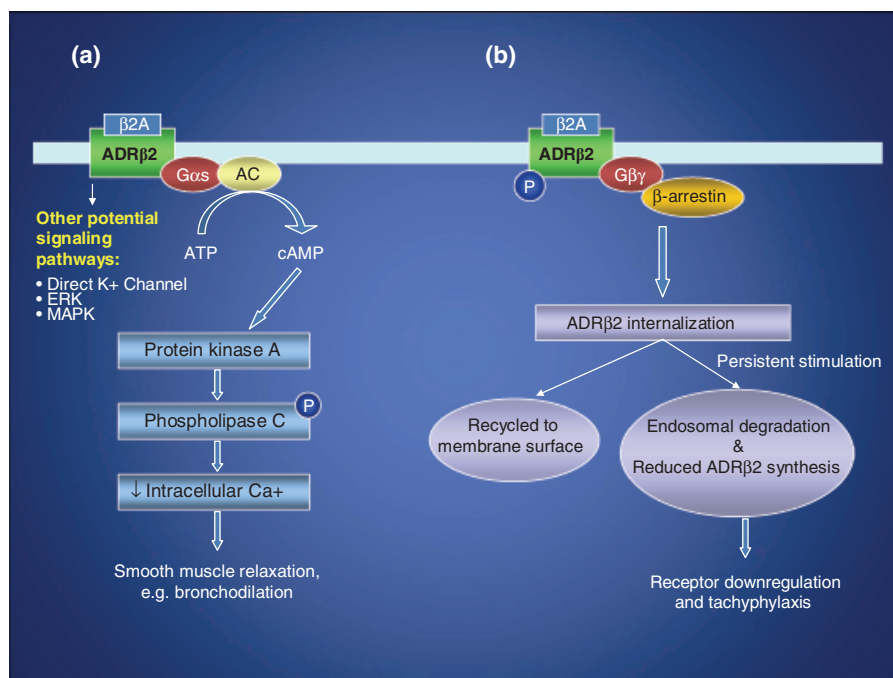


Fig. 1. Schematic diagram of conventional β_2 adrenergic receptor signalling pathway and receptor trafficking upon activation by β_2 adrenergic receptor agonists. (a) Binding of β_2 adrenergic receptor (ADRB2) agonists (β_2A) to ADRB2 results in binding to the α -subunit of stimulatory G protein ($G_{\alpha s}$), which leads to activation of adenylyl cyclase (AC) and a consequent increase in cAMP. cAMP activates protein kinase A (PKA), which induces phosphorylation (P) of various downstream proteins including phospholipase C and ADRB2 itself. Phospholipase C, together with other potential various mechanisms, e.g. direct K channels, reduces intracellular calcium (Ca^{2+}) thereby causing bronchodilation. (b) Phosphorylated ADRB2, however, is uncoupled from $G_{\alpha s}$ and binds to the β and γ subunits of inhibitory G protein ($G_{\beta\gamma}$). This terminates the receptor activation signal and stimulates binding to β -arrestin, causing receptor internalization [91]. Receptors are recycled back to the membrane upon prompt removal of the agonist. However, with persistent receptor activation, receptors are down-regulated through degradation of internalized receptors and reduced synthesis of new receptors [34–36].

and are the focus of intensive research. Detailed discussion of ADRB2 receptor trafficking and regulation are beyond the scope of this review. Briefly, short-term or immediate receptor desensitization results from receptor phosphorylation, binding of β -arrestin2, uncoupling of G-protein from the receptor and internalization of the receptors [33]. Internalized receptors are sequestered within endosomes from which they can be recycled to the cell surface (resensitization) or transferred to lysosomes for degradation. It is possible that proteins such as phosphodiesterase IV are translocated to the receptor microenvironment by binding to the β -arrestin2–ADRB2 scaffolding, where they metabolize locally generated cAMP and terminate the effects of receptor activation [29]. Complete reversal of desensitization may occur within hours of removal of the agonist. However, with persistent receptor stimulation, receptors are down-regulated through increased degradation, as well as decreased synthesis of new receptors [34–36]. Receptor desensitization and down-regulation manifest clinically as tachyphylaxis to ADRB2-agonist therapy.

β_2 -Adrenergic receptor gene

The gene encoding ADRB2, which was first cloned in 1987, contains one exon only and is located on chromo-

some 5q31–33 [37]. The gene is highly polymorphic with multiple single-nucleotide polymorphisms (SNPs). A detailed analysis reported in 1992 identified nine polymorphisms, including three SNPs within the coding region, which resulted in amino acid changes at positions 16 (ADRB2+46G > A), 27 (ADRB2+79C > G) and 164 (ADRB2+491C > T), often denoted as Arg16Gly, Gln27Glu and Thr164Ile, respectively [38]. More recently, a comprehensive analysis of the ADRB2 gene identified additional genetic variants within a 5.2 kb region of the gene [39] (Fig. 2). A total of 49 polymorphisms were identified, 21 of which were novel and had minor allele frequencies of < 10% in all ethnic groups. The analysis also showed that the 3'-UTR contained a poly-C repeat of variable length (11, 12, 13 or very rarely 14C), which is interrupted by polymorphisms at two different positions, giving rise to additional genetic variation (Fig. 3). The significance of these novel SNPs in determining asthma phenotypes and therapeutic responses to ADRB2 agonists is unknown.

The allele frequencies for the Arg16Gly and Gln27Glu SNPs vary with ethnicity [40, 41]. The reported allele frequency for Arg16 in the Caucasian, African American and Asian populations was 0.39, 0.50 and 0.40, respectively; while for Gln27 it was 0.57, 0.73 and 0.80, respectively [40]. The Thr164Ile SNP is rare, with the

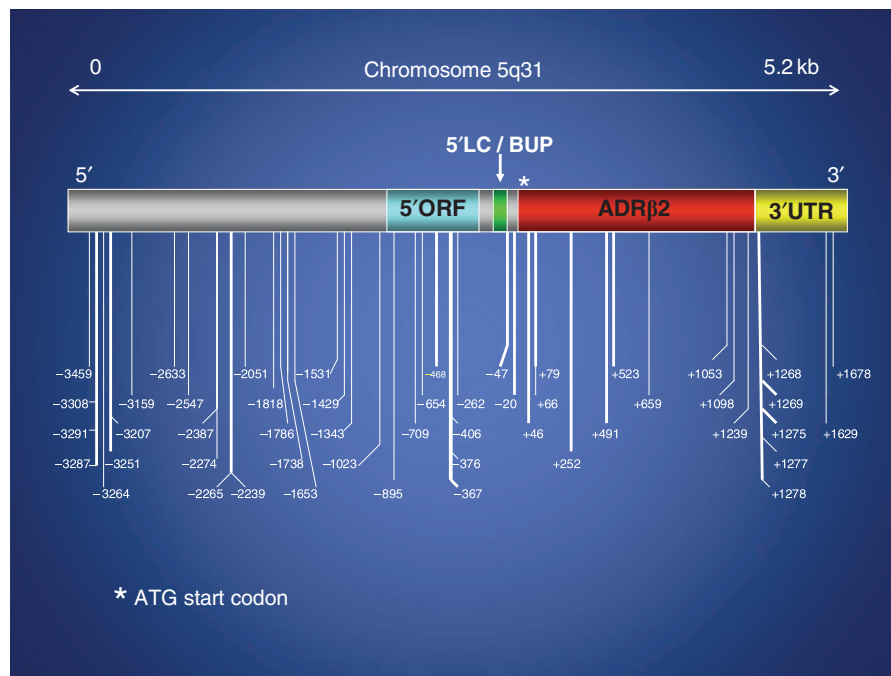


Fig. 2. Schematic diagram of β_2 adrenergic receptor gene polymorphisms. Adapted from Ortega *et al.* [91] (data from Hawkins *et al.* [39]).

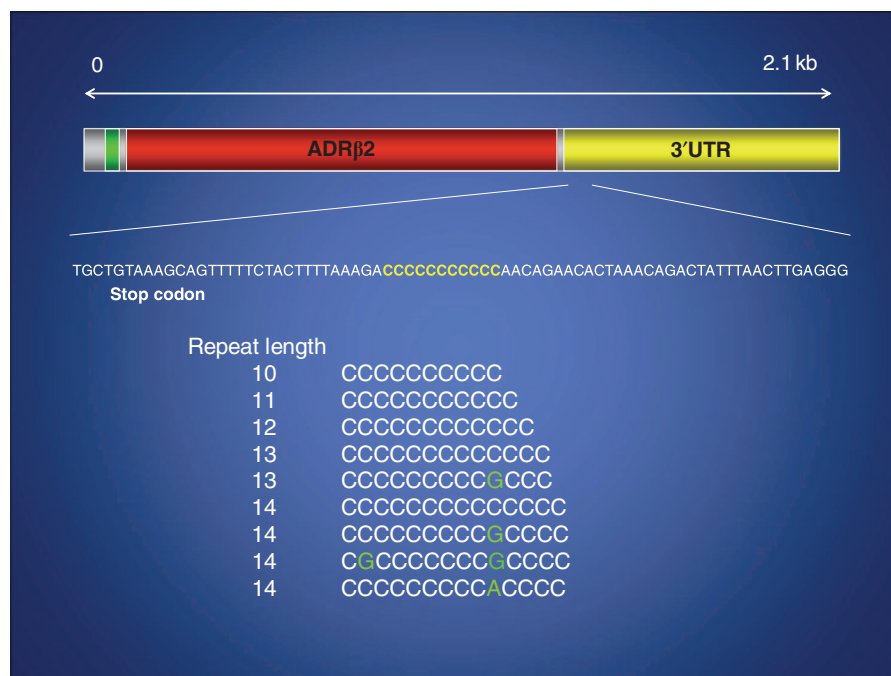


Fig. 3. Schematic diagram of 3'-untranslated region and poly-C region of β_2 adrenergic receptor gene. Adapted from Ortega *et al.* [91] (data from Hawkins *et al.* [39]).

frequency of heterozygosity being 3–5% in all populations studied [42, 43]. The allelic frequencies for these functional *ADR β 2* polymorphisms were similar in asthmatic patients and healthy control subjects [38, 44] but the extent to which they are expressed in severe asthma vs. milder forms of asthma is unknown.

The *ADR β 2* gene also codes for a separate translation regulatory protein, β -upstream peptide (BUP), within its 5' leader cistron region (Fig. 2). The last residue of this oligopeptide is polymorphic (Arg19Cys), with the Arg19 minor allele (–47C) resulting in lower basal *ADR β 2* expression *in vitro* [45]. Furthermore, promoter activity

was reduced for the most common haplotype that included the Arg19 polymorphism (–468G, –367C, –47C, –20C) [46, 47].

There is tight-linkage disequilibrium within the *ADRB2* gene [39, 48]. As a result, individuals homozygous for Arg16 are nearly always homozygous for Gln27. The Arg16/Gln27 haplotype is rare, occurring in <1% of the Caucasian population [42]. In addition, individuals homozygous for Arg16 and Gln27 are almost always homozygous for the Cys19 allele of the BUP Cys19Arg polymorphism [38, 39]. Consequent to this strong linkage disequilibrium, polymorphisms of the promoter and coding regions of the *ADRB2* gene have been organized by Drysdale and colleagues into 12 haplotypes, which are differentially expressed among ethnic groups [48] (Table 1).

Molecular effects of the adrenergic $\beta 2$ receptor gene polymorphisms

The Arg16Gly and Gln27Glu polymorphisms result in differential agonist-stimulated receptor down-regulation in transfected cell systems, including human airway smooth muscle cells [49, 50]. Significantly greater isoprenaline-induced receptor down-regulation was demonstrated in receptors with the Gly16 compared with the Arg16 genotype [49]. Conversely, down-regulation of receptors with the Glu27 genotype was attenuated in comparison with those with the Gln27 genotype [50]. When Glu27 is co-inherited with Gly16, phenotypic responses favour the Gly16 genotype [51, 52]. Gln27 influences the behaviour of cells with the Gly16Gln27 haplotype, which exhibits less acute and long-term desensitization as measured by cAMP levels [52].

The Thr164Ile polymorphism alters receptor-binding affinity, resulting in a significant reduction in basal receptor activity and *ADRB2* agonist-stimulated activation *in vitro* [51]. Ile164 receptors display a lower affinity for isoprenaline, adrenaline and noradrenaline but not for dobutamine or dopamine [51]. Initial studies also showed reduced receptor–G protein complex coupling and a significant reduction in adenylyl cyclase activity. Most *in vivo* data for the Thr164Ile polymorphism relate to the cardiovascular system, especially in the context of congestive heart failure (CHF). The prevalence of Ile164 was similar in patients with CHF and healthy control subjects [53, 54]. However, CHF patients who were heterozygous for Thr164Ile had lower exercise capacity compared with those without a polymorphic allele [55]. Other studies have demonstrated a more rapid progression of disease to death or heart transplantation in patients heterozygous for Thr164Ile [53, 54].

The *in vitro* effects of the Thr164Ile SNP may potentially be explained by the location of the polymorphism within the fourth transmembrane-spanning domain of the

Table 1. Drysdale haplotypes

Drysdale haplotype group	–1023G>A (5'-UTR)	–709C>A (5'-UTR)	–654G>A (5'-UTR)	–468C>G (5'-UTR)	–406C>T (5'-UTR)	–367T>C (5'-UTR)	–47T>C (Cys19Arg, BUP)	–20T>C (5'-UTR)	+46G>C (Arg16Gly)	+79C>G (Gln27Glu)	+252G>A (Ileu84Leu)	+491C>T (Thr164Ile)	+523C>A (Arg175Arg)	Frequency in Caucasians (%)	Frequency in African Americans (%)	Frequency in Asians (%)	Frequency in Hispanic- Latinos (%)
1	A	C	G	C	C	T	T	T	A	C	G	C	C	0.7	25	12.5	10.0
2	A	C	G	G	C	C	C	C	G	G	G	C	C	48.3	6.3	10.0	26.7
3	G	A	A	C	C	T	T	T	A	C	G	C	C	0.7	0.0	0.0	0
4	G	C	A	C	C	T	T	T	A	C	G	C	C	33	29.7	45.0	40.0
5	G	C	A	C	C	T	T	T	G	C	G	C	C	1.4	0	0	0
6	G	C	G	C	C	T	T	T	G	C	A	C	A	13.2	31.3	30.0	13.3
7	G	C	G	C	C	T	T	T	G	C	A	T	A	1	1.6	0	3.3
8	G	C	A	C	C	T	T	T	A	C	A	C	A	0.7	0	0	0
9	A	C	G	C	T	T	T	T	A	C	G	C	C	0	4.7	0	0
10	G	C	G	C	C	T	T	T	G	C	A	C	C	0.7	0	0	3.3
11	G	C	G	C	C	T	T	T	G	C	G	C	C	0.3	0	2.5	0
12	A	C	G	G	C	T	T	T	A	C	G	C	C	0	1.6	0	3.3

Data from Drysdale *et al.* [48].
UTR, untranslated region; BUP, β -upstream peptide.

receptor, which is predicted to interact with the β -carbon hydroxyl groups of isoprenaline and noradrenaline, but not present in dobutamine or dopamine [51, 56]. The β -carbon hydroxyl group is also common to β -agonists such as terbutaline, salbutamol, salmeterol and formoterol. Increases in heart rate, myocardial contractility and venodilation following terbutaline infusion were blunted in patients with congestive cardiomyopathy, as well as healthy control subjects, who were heterozygous for Thr164Ile [57, 58]. Therefore, it is plausible that bronchodilator responses to these drugs are altered in individuals carrying this genetic variation. In a single *in vitro* study, the affinity of Ile164 receptor for isoprenaline, terbutaline and formoterol was reduced significantly by approximately two- to threefold compared with wild-type Thr164 receptor in Chinese hamster fibroblasts [59]. Binding of salmeterol or salbutamol to Ile164 receptors was also reduced (1.2- and 1.6-fold, respectively) but this did not reach statistical significance. Nonetheless, all *ADR β 2* agonists studied showed considerable reduction in maximal stimulation of adenylyl cyclase. Similarly, impaired signal transduction efficiencies were noted for all *ADR β 2* agonists but to a lesser extent for formoterol and salmeterol. The duration of action of salmeterol was halved in Ile164 receptors compared with Thr164, possibly explained by altered agonist-binding properties. Of note, such an effect appears agonist-specific because the duration of action of formoterol and isoprenaline was similar for both Ile164 and Thr164 receptors under the same experimental conditions. However, there have been no *in vivo* studies examining the interaction between Thr164Ile and *ADR β 2* agonists commonly used in the respiratory system. A clinical study looking at the associations among Thr164Ile polymorphism, asthma severity and therapeutic responses to *ADR β 2* agonists would be important but may be hindered by the relative rarity of the minor allele in any ethnic group.

Expression of *ADR β 2* is altered by polymorphisms within the promoter region, as well as the 3'-UTR poly-C repeats. Caucasians with the Arg16 genotype represent three different haplotypes defined by the length of the poly-C repeats, with haplotype frequencies ranging from 14% to 43% [39]. An *in vitro* study showed lower mRNA and receptor expression, more extensive mRNA degradation and a greater tendency for *ADR β 2* down-regulation in cells transfected with the Arg16-11C haplotype, as compared with the other two haplotypes [60]. Such differences in Arg16 genotype may result in important phenotypic variation in the *in vivo* responses to *ADR β 2* agonists used in the treatment of asthma, and may in part, explain the discrepancies in clinical studies investigating the relationship between treatment responses and Arg16-Gly polymorphism alone. More recently, Panebra and colleagues demonstrated haplotype-dependent differences in cells surface *ADR β 2* expression and agonist-

induced down-regulation. The *in vitro* differences in haplotypes were driven not only by common variations within the coding region but also by variations in the poly-C repeat [16]. More importantly, two haplotypes showed high *ADR β 2* expression and less agonist-induced down-regulation compared with other six common haplotypes, which may help to predict those likely to have better bronchodilator response to intermittent administration of *ADR β 2* agonists and minimal tachyphylaxis with chronic therapy. To date, variations of the poly-C repeat have yet to be investigated in clinical studies, and it is highly likely that assessment of the effects of poly-C polymorphism in conjunction with other *ADR β 2* polymorphisms or haplotypes would better predict therapeutic responses to *ADR β 2* agonists. Further studies, preferably clinical trials, are required to determine the functional significance of poly-C polymorphism and its interactions with other known SNPs.

Clinical pharmacogenetic studies

Effect of adrenergic β_2 receptor gene polymorphisms on acute bronchodilator response to short-acting β -agonists

In 1997, Martinez and colleagues conducted one of the earliest studies examining the pharmacogenetic relationship between *ADR β 2* genetic variants and pharmacological responses to acute SABA therapy [23]. A total of 269 children with mild asthma were genotyped for the Arg16-Gly and Gln27Glu polymorphisms. Spirometry was performed before and after a single dose of salbutamol and a positive bronchodilator response was defined as > 15.3% increase in FEV1. Arg16 homozygotes were 5.3 times and Arg16Gly heterozygotes were 2.3 times more likely to show a positive response to salbutamol, and had higher predicted FEV1 values compared with the Gly16 homozygotes. There was no association with the Gln27Glu polymorphism. The findings were similar in asthmatic and non-asthmatic children, and in children from Caucasian or Hispanic backgrounds.

An association between higher post-bronchodilator FEV1 values and Arg16 homozygosity was also demonstrated in a study involving 707 mild-to-moderate asthmatic children enrolled in the Childhood Asthma Management Programme, in which family-based genetic associations for eight *ADR β 2* polymorphisms were analysed [24]. In that study, polymorphisms at positions -654 (promoter) and +523 (coding region) were also associated with greater post-bronchodilator FEV1. However, the findings relating to the -654 and +523 polymorphisms have not been replicated in other studies.

Similar associations of the Arg16Gly genotype with a response to acute SABA therapy were observed in other smaller studies [18, 21, 25]. In a study of 16 clinically stable, moderate asthmatics, as defined by lung function,

the bronchodilator response following *oral* administration of salbutamol (resulting in equivalent plasma concentrations) was greater and more rapid in Arg16 homozygotes than in those who were homozygous for Gly16 [22].

Conversely, a study by Taylor and colleagues did not show any significant genotypic associations with bronchodilator response in 176 patients with mild-to-moderate asthma, who had been successfully weaned off all *ADRβ2* agonist and ICS therapy [61].

Potential ethnic influences on genetic differences in bronchodilator responses were the primary focus of the Genetics of Asthma in Latino Americans study [62]. Based on United States statistics, there is a fourfold difference in the prevalence of asthma between Puerto Ricans and Mexicans, representing the subpopulations with the highest and lowest asthma morbidity and mortality, respectively [63, 64]. A greater bronchodilator response was observed in Puerto Ricans who were homozygous for Arg16 but this finding was limited to those with baseline FEV1 <80% of predicted. There was no association between Arg16 genotype and drug responsiveness among Mexicans with asthma. Although allele frequencies for Arg16 were similar in the two ethnic groups, the pattern of linkage disequilibrium differed noticeably, which may be a potential explanation for the findings. However, environmental and socio-behavioural differences are important confounders that were not examined in this study.

Effect of adrenergic β2 receptor gene polymorphisms on clinical responses to regular use of short-acting β-agonists

The regular use of SABA does not improve asthma control and may even lead to clinical deterioration [65]. The precise mechanism for this is unclear but both agonist-induced receptor down-regulation [66, 67] and *ADRβ2* agonistic pro-inflammatory effect have been implicated. The immunomodulating effects of *ADRβ2* agonists require further investigation and there are emerging data suggesting pro-inflammatory effects associated with persistent *ADRβ2* activation resulting in increased airway inflammation and hyperresponsiveness [68, 69]. Nguyen *et al.* [69] demonstrated that *ADRβ2* signalling is required for the development of mucin production, airway hyperresponsiveness and inflammatory cellular infiltrate in a murine model of asthma. As such, *ADRβ2* agonism, rather than agonist-induced receptor down-regulation, may represent an alternative mechanism for adverse effects related to chronic *ADRβ2* agonist therapy. Receptor down-regulation and *ADRβ2* signalling may be influenced by certain *ADRβ2* polymorphisms, namely Arg16-Gly, Gln27Glu and Thr164Ile. Current international clinical practice guidelines do not recommend the use of regular SABA therapy for long-term control of asthma symptoms. Predisposing genetic influences on clinical responses to therapy become highly relevant and have

major implications during periods of clinical instability, when the dosage and frequency of *ADRβ2* agonist use are escalated. This may occur during exacerbations in many individuals with mild or moderate asthma, as well as in those with severe persistent disease.

An early pharmacogenetic study showed no association between *ADRβ2* genotype and overall asthma control with regular SABA therapy [70]. Subjects with mild-to-moderate asthma were randomized to fenoterol 400 µg q.i.d. or matching placebo over 24 weeks in a double-blind, cross-over study. Usual maintenance therapy with ICS and/or cromoglycate was continued at constant doses. Data from 60 subjects did not show any differences in PEFR, FEV1, additional bronchodilator use, bronchodilator response or asthma symptom scores, between those with Arg16Gly or Gln27Glu polymorphisms. However, bronchial hyperresponsiveness to methacholine during regular SABA therapy was increased in subjects who were homozygous or heterozygous for Arg16, compared with Gly16 homozygotes. However, these results must be interpreted with caution, given that SABA rescue therapy was permitted for all patients throughout the study, and it is unclear what proportions of the subjects were using corticosteroids or cromoglycate.

In contrast, two studies by Israel *et al.* [19, 20] demonstrated significant increases in adverse events among Arg16 homozygotes using SABA regularly, compared with those using *ADRβ2* agonists as needed, or subjects who were homozygous for Gly16. The first study was a retrospective genotype analysis of 190 mild asthmatics randomized to regular or as-needed salbutamol over 16 weeks. A decline in morning PEFR was noted in the Arg16 homozygotes during regular SABA treatment, and this change was more prominent during the 4-week wash-out period [20]. At the end of the study, morning PEFR for Arg16 homozygotes was significantly lower by 30.5 ± 12.1 and 23.8 ± 9.5 L/min, compared with those using salbutamol as required salbutamol, and Gly16 homozygotes using regular salbutamol, respectively. There were no clinically significant genotype differences for the Gln27Glu polymorphism. The mechanism underlying the observed deterioration during the wash-out phase following regular salbutamol use was unclear, but may be secondary to a potential masking phenomenon related to the bronchodilating effect of regular salbutamol.

The potential deleterious effect of the Arg16 genotype was again demonstrated by Israel and colleagues in a prospective, genotype-stratified, placebo-controlled, cross-over study of 78 subjects with mild asthma [19]. Improvement in morning PEFR was observed for Arg16 homozygotes during the run-in period, when salbutamol use was minimized and ipratropium was used as an alternative rescue therapy. Regular salbutamol use during the treatment phase was associated with a fall in morning PEFR and an adverse effect on asthma symptom score,

lung function and rescue medication use in Arg16 homozygotes. In contrast, Gly16 homozygotes benefited from regular salbutamol and showed significant improvements in the clinical parameters.

A higher frequency of exacerbations has been reported among mild-to-moderate asthmatics who were homozygous for Arg16, when chronically dosed with salbutamol as compared with as-needed therapy [26]. There was no difference in treatment-related outcomes between the Gln27 and Glu27 genotypes.

Small and colleagues have suggested that the dynamic theory of receptor kinetics may provide a possible mechanism for the contrasting genotype associations between Arg16Gly and clinical outcomes during acute and chronic dosing with SABA (Fig. 4) [42, 43]. In a static model, receptors are not regulated by endogenous agonists. As such, Gly16 receptors will undergo maximum desensitization and down-regulation when exposed to exogenous $ADR\beta 2$ agonists, as predicted by the *in vitro* data. However, many G protein-coupled receptors, including $ADR\beta 2$, are under constant modulation by endogenous agonists [71]. In the dynamic model, Gly16 receptors are maximally down-regulated by endogenous catecholamines, and therefore show a reduced response on first acute exposure to exogenous $ADR\beta 2$ agonists, with no scope for further down-regulation or tachyphylaxis with chronic exposure. In Arg16 homozygotes, the receptors are less susceptible to endogenous agonist-induced down-regulation, which would explain the superior response to acute $ADR\beta 2$ agonist therapy compared with Gly16

homozygotes. Arg16 receptors may undergo further down-regulation with prolonged or chronic $ADR\beta 2$ agonist therapy, giving rise to clinical tachyphylaxis.

Effect of adrenergic $\beta 2$ receptor gene polymorphisms on clinical response to regular long-acting β -agonists

The study by Taylor *et al.* [26] that examined the relationship between asthma exacerbations and regular use of salbutamol also included a cohort of patients who were randomized to regular treatment with the LABA, salmeterol. There were no genotype-related adverse effects in the subgroup treated with salmeterol. This finding contradicted the results of a cross-sectional survey by Palmer *et al.*, [72] which showed an increased likelihood of exacerbations in children and young adults with mild asthma who were treated with salmeterol, but not in those using salbutamol as required. Of note, asthma exacerbations were defined as absence from school, the use of oral corticosteroids and hospital admissions, and so relied on parental recall, which may have biased the study outcomes.

A decline in PEFR in mild-to-moderate asthmatics who were homozygous for Arg16 was noted in the study by Wechsler and colleagues, which included patients from the salmeterol or corticosteroid (SOCS) and salmeterol $\pm$ inhaled corticosteroid (SLIC) trials [27]. These were small studies and the results have not been confirmed by larger prospective trials.

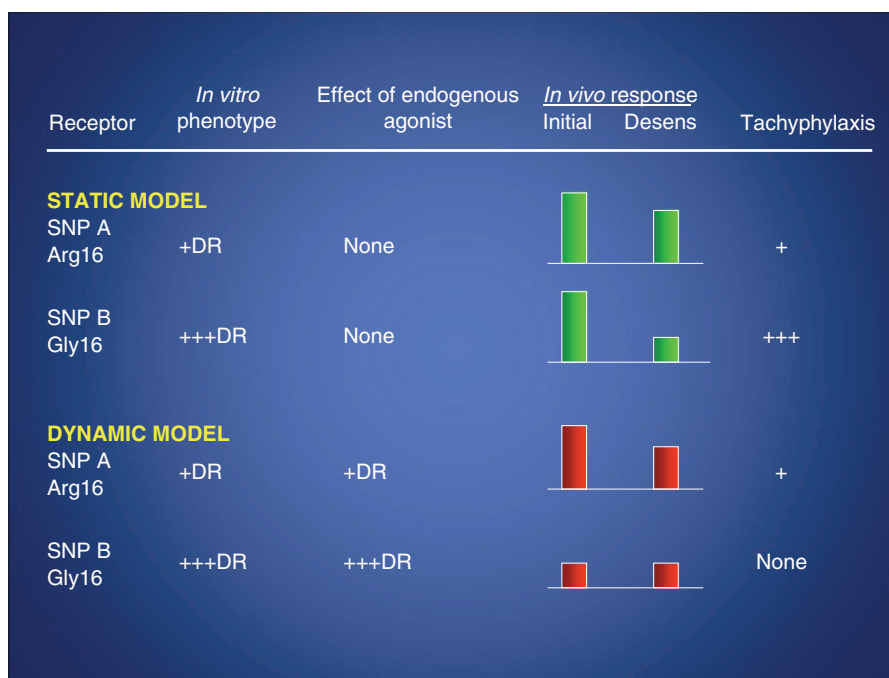


Fig. 4. Schema of the Static and Dynamic Theories of Receptor Regulation. Coloured bars represent arbitrary physiological responses. Adapted from Small *et al.* [43]. SNP, single-nucleotide polymorphism; DR, down-regulation.

The SOCS trial was a 28-week randomized, placebo-controlled trial, comparing salmeterol with ICS monotherapy in subjects with persistent asthma [73]. The authors concluded that there was a significant risk of loss of asthma control when patients with persistent asthma, which was well controlled on a low dose of corticosteroid, were switched to salmeterol monotherapy. The SLIC trial examined the effect of adding salmeterol to stable or decreasing doses of ICS in patients with asthma that was inadequately controlled by ICS monotherapy [74]. The study demonstrated that the ICS dose could be halved, without significant risk of clinical deterioration, in patients whose symptoms improved following the addition of salmeterol. However, cessation of ICS could not be recommended due to a significant deleterious effect on asthma control. Retrospective genotype analysis of eight *ADRB2* SNPs was performed for 96 patients from the SOCS trial and 78 patients from the SLIC trial [27]. In the SOCS trial, morning PEFR declined by 29.9 L/min ($P=0.04$) in Arg16 homozygotes treated with salmeterol compared with placebo. In contrast, morning PEFR improved by 21.5 L/min ($P=0.04$) in Gly16 homozygotes receiving salmeterol monotherapy.

In the SLIC trial, deterioration was also noted in the Arg16 homozygotes who received concurrent salmeterol and ICS therapy. The apparent genotype-related differences in these two trials must be interpreted with caution, as there were limitations to these studies. First, patients continued to use SABA as the preferred rescue therapy and a potential confounding effect cannot be excluded. Second, maintenance ICS therapy was stopped at the time of randomization to salmeterol monotherapy in the SOCS trial. The small numbers of patients in these studies also needs to be considered when interpreting the results.

Genotype analysis was performed in another study comparing the safety and efficacy of fluticasone/salmeterol with that of montelukast, in 183 patients with moderate asthma that was inadequately controlled on SABA alone. Sustained improvements in symptoms and lung function were reported for all patients treated with fluticasone/salmeterol over 12 weeks, regardless of genotype (Arg16Gly, Gln27Glu, Thr164Ile and BUP Cys19Arg) [75]. This finding was confirmed by one retrospective and three prospective studies designed to examine the pharmacogenetic relationship between *ADRB2* polymorphisms and use of salmeterol with or without ICS [76–79].

In the LARGE study [79], 87 mild-to-moderate asthmatics stratified by Arg16Gly genotypes were randomized to ICS and ipratropium with or without salmeterol following successful LABA withdrawal. The improvement in morning PEFR with salmeterol therapy was similar for Arg16 and Gly16 homozygous asthmatics. Of note, responsiveness to methacholine was unchanged in Arg16 homozygotes but methacholine PC20 was over twofolds higher in Gly16 homozygotes treated with salmeterol

compared with placebo. The genotype-specific difference in methacholine responsiveness was significant. This highlights not only the bronchodilating effect of LABA therapy but also desensitization of bronchoprotective effects with chronic LABA use. Therefore, the chronic use of LABA in Arg16 homozygous asthmatics may lead to a greater risk of deterioration in asthma control or exacerbation compared with Gly16 homozygous asthmatics. However, the observed exacerbation rates for Arg16 and Gly16 homozygotes were not significantly different in the LARGE trial. *Post hoc* subgroup analysis of the African-American participants showed significant improvement in PEFR with salmeterol use compared with placebo in Gly16 homozygotes ($n=8$) but not in asthmatics homozygous for Arg16 ($n=9$). The genotype-specific differences had borderline statistical significance ($P=0.07$) and require confirmation in larger population.

In 2007, Bleecker *et al.* [78] reported that *ADRB2* polymorphisms were not associated with differential clinical responses to a variety of LABA/ICS treatment regimens. These regimens included budesonide/formoterol as a single maintenance and reliever therapy, adjustable or fixed doses of budesonide/formoterol with SABA as required and fixed doses of fluticasone/salmeterol with SABA as required. Rates of exacerbations, FEV1, frequencies of nocturnal awakening and use of rescue medication were similar for patients with different genotypes that included 11 polymorphisms in the promoter and coding regions of the *ADRB2* gene. Although this was a large and important pharmacogenetic study that included a total of 2655 patients, it did have limitations. The total *ADRB2* agonist (formoterol or salmeterol) dose was modest and patients requiring frequent doses of SABA for symptomatic relief were excluded. The genotype analysis was performed *post hoc* and was not part of the original study design. A more recent prospective study by Bleecker *et al.* [80] again demonstrated that asthmatics stratified by Arg16Gly genotype showed similar clinical responses to salmeterol alone, or in combination with fluticasone. The study sample size provided >90% power at demonstrating non-inferiority, defined as mean change of 28.5 L/min or more in morning PEFR from baseline, between Arg16 and Gly16 homozygous genotypes in the respective treatment arms. Overall, the mean changes in morning PEFR were not statistically different between Arg16 and Gly16 homozygotes within each treatment arm. Although there was greater number of asthma exacerbations in the group treated with salmeterol alone ($n=7/272$) compared with the group treated with fluticasone and salmeterol ($n=1/272$), this was not statistically significant. The exacerbation rates following treatment randomization were similar in Arg16 and Gly16 homozygotes. Exacerbations were significantly higher during the run-in phases of the study in the Arg16 homozygotes ($n=10$) compared with Gly16 homozygotes ($n=2$) based on an intention-to-treat

analysis. However, of these, only five occurred in Caucasian asthmatics and there was no difference in exacerbations by genotype (Arg16 homozygotes, $n=4$; Gly16 homozygotes, $n=1$). Genotype-attributable risk of asthma exacerbations in other ethnic groups requires further clarification. However, this study was again conducted in individuals with mild asthma who were treated with SABA alone before enrolment, and subjects requiring additional maintenance therapy such as LABA, corticosteroids or leukotriene antagonists were excluded. Therefore, the apparent safety and efficacy of salmeterol or formoterol in combination with ICS should not be generalized to patients with at risk genotypes (e.g. Arg16Gly) requiring high-dose LABA or frequent SABA, such as those with severe persistent, or poorly controlled asthma.

Future directions

Haplotype analysis

The effect of a single SNP may be negated by other polymorphisms and it is the haplotype that determines the overall characteristics of the receptor. Haplotype analysis should therefore provide a better indication of clinical response to β -agonists. There are limited haplotype data for the *ADRB2* gene but the most detailed analysis was performed by Drysdale *et al.* [48], using transfected HEK293 cell lines. Gene transcription and receptor expression were significantly higher in cells homozygous for Drysdale haplotype 2 (Table 1) than in those homozygous for haplotype 4, the two most frequent haplotypes in the Caucasian population. Of note, the observed increase in *ADRB2* expression in HEK293 cells was not accompanied by a proportionate increase in agonist-stimulated adenylyl cyclase activity. However, this haplotype data did not take into account the potentially crucial confounding effect of the poly-C repeat polymorphism. Furthermore, the significance of these haplotypes in cell types that are physiologically relevant to asthma, such as smooth muscle cells, warrants further investigation.

Although multiple *ADRB2* polymorphisms have been genotyped for a number of clinical trials, the vast majority of these studies were limited to retrospective analysis of the relationship between genotypes and responses to asthma therapy, and were inadequately powered for haplotype analysis. The *in vitro* results of Drysdale and colleagues are, however, consistent with their *in vivo* findings on the bronchodilator response to salbutamol, which was greatest for haplotype 2 and least for haplotype 4, in an asthmatic population [48]. In contrast, another study that included 138 mild-to-moderate asthmatics showed no haplotype association with changes in FEV1 [61]. These results, however, could be explained by differences in patient selection criteria and the study protocol.

In the study of Drysdale and colleagues, patients were selected for positive bronchodilator responses at recruitment and prior exposure to an *ADRB2* agonist was permitted. In the second study, patient recruitment was based on diagnosis of asthma by a physician, and bronchodilator responses were assessed after successful, sequential withdrawal of all *ADRB2* agonist and ICS therapy [61].

The apparent clinical effects and associations observed for any given SNP such as Arg16 may also be attributable to other polymorphisms that are in tight-linkage disequilibrium with Arg16. Haplotype analyses would provide better insights into other polymorphisms that may influence an individual's response to *ADRB2* agonist therapy. However, the inheritance of heterozygous haplotypes adds to the genetic diversity and complexity, and a complex study design would almost certainly be required to determine the functional and clinical significance of such haplotypic variations.

One approach would be to obtain peripheral blood mononuclear cells (PBMC) from well-phenotyped asthmatics of known *ADRB2* haplotypes and haplotype pairs and examine for *in vitro* changes in receptor expression and activity before and after successful SABA and LABA withdrawal while monitoring asthma symptom control, PEF and lung function. In addition, parallel *in vitro* experiments should be conducted using asthmatic PBMC to examine for differences in receptor expression and activation (e.g. cAMP measurements) following prolonged incubation with commonly used *ADRB2* agonists to differentiate haplotype-specific vs. *ADRB2* agonist-specific effects. Differences observed with haplotypes and specific *ADRB2* agonist *in vitro* should be confirmed in a prospective clinical study in which asthmatics of identical haplotypes are randomized to different LABA and their clinical status is monitored. It would be important to include both mild and severe asthmatics in these studies because haplotype-specific adverse events may only become apparent in those with a higher use of *ADRB2* agonists.

Differential effects of adrenergic β_2 receptor agonists

The commonly used β -agonists differ significantly in their chemical structures, resulting in varied mechanisms of action and pharmacological properties. It is unclear if the various *ADRB2* agonists interact differentially with genetic variants [81, 82]. Moore *et al.* [82] demonstrated significant disparities in agonist-induced receptor down-regulation for various *ADRB2* agonists. The greatest receptor down-regulation was observed in cells treated with formoterol, which has the highest intrinsic activity of all the commercially available SABA or LABA [82]. Conversely, surface receptor density was relatively preserved in cells treated with salmeterol, which has a relatively low

intrinsic activity. Selected clinical studies have shown a greater tendency for clinical tachyphylaxis in Arg16 homozygous asthmatic patients using regular SABA or LABA therapy [17, 25, 83, 84]. In a retrospective analysis of six randomized, double-blind, placebo-controlled studies, in which LABA were administered for 1–2 weeks, tachyphylaxis was greater in Arg16 homozygotes treated with formoterol as compared with salmeterol [83]. However, the results were by no means conclusive, because these pharmacogenetic studies were performed on small numbers of patients, and there were critical differences in primary outcomes (e.g. bronchodilatation, bronchoprotection or reversal of stimulant-induced airway constriction), study protocols, drug doses and choice of airway stimulant (e.g. methacholine or adenosine monophosphate). It is, however, possible that the pharmacogenetic effects of *ADRB2* polymorphisms are agonist specific. The alternative hypothesis is that all *ADRB2* agonists have the same therapeutic phenotype but that individuals carrying certain polymorphisms are at greater risk of poor outcomes following the use of *ADRB2* agonists. Both *in vitro* and clinical studies are important in order to assess the interactions between *ADRB2* polymorphisms/haplotypes and responses to *ADRB2* agonists with varying structures and activities.

Implications for the treatment of patients with severe asthma or acute exacerbations in a hospital setting

The available clinical data for associations between *ADRB2* polymorphism and asthma are almost exclusively derived from patients with mild or moderate asthma, which was managed in a community setting. The significance of genetic variation in the *ADRB2* has rarely been addressed in patients with severe asthma. Weir and colleagues genotyped 84 non-atopic, non-asthmatic individuals, 86 mild-to-moderate asthmatics and 81 individuals with fatal or near-fatal asthma for the Arg16Gly and Gln27Glu polymorphisms [40]. They concluded that there was no significant association between these *ADRB2* polymorphisms and fatal or near-fatal asthma. However, the Gly16/Gln27 haplotype was more prevalent in patients with moderate asthma compared with those with mild asthma [40]. Another study by Holloway and colleagues also concluded that there was no association between Arg16Gly or Gln27Glu and a diagnosis of asthma *per se*, but that there may be a potential role for Gln27Glu in the pathogenesis of severe asthma [44]. A third study of 22 moderately severe asthmatics demonstrated significantly greater desensitization to bronchodilatation following regular therapy with formoterol in patients who were homozygous for Gly16 [25]. This finding contradicted the published results for mild asthmatics. However, the results from this study were significantly undermined by the

small number of patients, with only four being homozygous for Arg16.

Two studies that examined the relationship between *ADRB2* polymorphisms and clinical responses during acute asthma exacerbations yielded conflicting results [85, 86]. Martin and colleagues [86] studied the clinical responses to inhaled *ADRB2* agonist in 148 children with mild-to-moderate exacerbations of asthma, and genotyped these children for Arg16Gly and Gln27Glu polymorphisms. The time taken to reduce the frequency of salbutamol use from 20-min to 1-, 2- and 4-h intervals was used as a surrogate marker of response, with shorter times indicating a better response. While there was no association between genotypes and the severity of exacerbations, Gln27 homozygotes were identified as poor responders, requiring a significantly longer time for decreased frequency of *ADRB2* agonist use. There was no association between any clinical parameter and the Arg16Gly polymorphism. Contrary to this, Carroll and colleagues genotyped 37 children who were admitted to the intensive care unit (ICU) with severe exacerbations of asthma, and reported more rapid responses in children who were homozygous for Gly16 [85]. In this study, Gly16 homozygotes had shorter stays in the ICU, shorter durations of continuous nebulized albuterol therapy and required less supplemental oxygen. The different results from these studies may be explained by differences in the severity of exacerbations in the two study cohorts.

Gene–gene interactions with the glucocorticoid receptor and mutations downstream from adrenergic β 2 receptor activation

Combinations of *ADRB2* agonists and corticosteroids are frequently used in the treatment of severe asthma and asthma exacerbations. Synergistic interactions between *ADRB2* and GR activation are well documented. Corticosteroids increase *ADRB2* expression and could potentially protect against *ADRB2* agonist-induced receptor down-regulation. *ADRB2* agonists may enhance GR function by enhancing nuclear translocation and co-factor activation. The GR gene is also highly polymorphic and multiple splice variants and epigenetic modifications have been described. Mathews and colleagues demonstrated that defective histone acetylation was associated with impaired nuclear localization of GR in response to high-dose corticosteroids [87]. To date, there have been no *in vitro* studies examining the functional effects of *ADRB2* polymorphisms on *ADRB2* expression or regulation in cells treated with corticosteroids.

There is an even greater deficit in our understanding of the interactions between polymorphisms in the *ADRB2* and GR genes. Although many of the clinical trials discussed previously attempted to examine the significance of *ADRB2* polymorphisms in patients co-treated

with corticosteroids, definitive conclusions could not be drawn due to the diversity in patient selection and study design, including variations in the type and dose of LABA and corticosteroid. Furthermore, these studies were mostly performed in patients with mild or moderate asthma and the findings may not be applicable to patients with severe asthma, who are treated with high doses of *ADR β 2* agonists, despite the potential protective effects of corticosteroids.

In addition to associations between *ADR β 2* polymorphisms and differential receptor down-regulation, genetic variations in proteins involved in the complex process of receptor trafficking may also influence down-regulation. For example, G protein receptor kinase 5 is involved in the process of receptor desensitization through phosphorylation. A mutation resulting in a single amino acid substitution, which occurs almost exclusively in African Americans, has been shown to alter the rate of phosphorylation and receptor internalization [88]. Whether this contributes to variability in treatment response is unknown. Various functional polymorphisms have been identified in substrates or effectors that are downstream of *ADR β 2* activation and are potentially involved in the cross-talk between *ADR β 2* agonists and corticosteroids (e.g. adenylyl cyclase 9) [89, 90].

Trafficking of *ADR β 2* is highly regulated and an understanding of the regulatory elements in the signalling pathways, including phosphorylation and ubiquitination, is likely to be important in deciphering the significance of the associations between *ADR β 2* polymorphisms and *ADR β 2* down-regulation. Although our understanding of *ADR β 2* signalling and trafficking has advanced considerably in the last decade, real insights into the genetic aspects of the critical pathway components are relatively scarce. The effect of gene-to-gene interactions on clinical responses to *ADR β 2* agonists is an area that has been

poorly researched. As our knowledge in these areas advances, a pharmacogenomic approach may be more appropriate for better prediction of *in vitro* and immediate and long-term clinical response to *ADR β 2* agonist therapy.

Conclusions

ADR β 2 agonists are the most frequently prescribed drugs for the management of airways obstruction. While *ADR β 2* agonists can be used safely and effectively in most patients, there is evidence to suggest that certain minority groups are at risk of adverse events related to their use. In addition to the potential psychosocial, behavioural and environmental factors contributing to severe or poorly controlled asthma, genetic predisposition is an important consideration. Despite differences in patient selection and study design, the evidence implicating *ADR β 2* polymorphisms in altered therapeutic responses to commonly used *ADR β 2* agonists is substantial and warrants further investigation.

Future studies aimed at defining the role of *ADR β 2* polymorphisms in severe asthma should be geared towards determining the *in vitro* and *in vivo* effects of *ADR β 2* haplotypes rather than genotypes. It is also critical to investigate whether the effect of a given haplotype is also *ADR β 2* agonist specific and whether corticosteroids influence the impact of specific haplotypes. Gene-gene interactions between *ADR β 2* polymorphisms and genetic variants of the GR or proteins downstream of *ADR β 2* activation should also be explored. It is important that these key questions be studied in depth, especially in patients with severe or unstable asthma, who are most at risk of undesirable outcomes due to their requirement for high-dose *ADR β 2* agonist treatment.

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