Polymorphism of the $\beta_2$-adrenoceptor and the response to long-term $\beta_2$-agonist therapy in asthma

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Polymorphism of the $\beta_2$-adrenoceptor and the response to long-term $\beta_2$-agonist therapy in asthma. R.J. Hancox, M.R. Sears, D.R. Taylor, ©ERS Journals Ltd 1998.

ABSTRACT: Polymorphisms affecting amino acids 16 and 27 of the $\beta_2$-adrenoceptor alter receptor regulation in vitro. Whether these polymorphisms alter the response to $\beta_2$-agonist therapy in asthma is unknown. In a previous study of 64 asthmatics, most experienced a deterioration in asthma control during regular inhaled $\beta_2$-agonist (fenoterol) treatment, while a minority improved. We have determined the $\beta_2$-adrenoceptor genotypes in these subjects, to establish whether changes in asthma control during the earlier study were influenced by $\beta_2$-adrenoceptor polymorphism.

The genotypes coding for amino acids 16 and 27 were identified in 60 subjects using allele-specific polymerase chain reaction. The effects of regular $\beta_2$-agonist treatment on asthma control were compared between genotypes.

There was no association between genotype and change in overall asthma control during regular $\beta_2$-agonist treatment. Only two of 10 markers of asthma control showed changes that were significantly associated with genotype: subjects homozygous for glycine at position 16 had no increase in bronchial responsiveness to methacholine during regular treatment; subjects homozygous for glutamic acid at position 27 had no increase in evening peak expiratory flow rates during regular treatment. These differences are the opposite of those that would have been predicted by the results of in vitro studies.

In these subjects, the deleterious response to regular inhaled $\beta_2$-agonist treatment was not related to $\beta_2$-receptor polymorphism. Eur Respir J 1998; 11: 589–593.

In 1990 we reported a randomized controlled trial in which the administration of regular inhaled $\beta_2$-agonist therapy was associated with a deterioration in asthma control in a majority of subjects when compared to as-required $\beta_2$-agonist treatment [1]. There were more asthma exacerbations, reductions in the forced expiratory volume in one second (FEV1) and morning peak expiratory flow rates (PEFRs) and increased bronchial responsiveness to methacholine during regular fenoterol therapy [2].

The mechanism responsible for the deterioration in asthma control during regular $\beta_2$-agonist therapy is unknown, but agonist-induced down-regulation of $\beta_2$-adrenoceptor function is one possible explanation. In our study there was no evidence of loss of the acute bronchodilator response to $\beta_2$-agonist after prolonged therapy, suggesting that down-regulation of airway smooth muscle $\beta_2$-receptors was not a significant problem [2]. However, tolerance to the nonbronchodilating properties of inhaled $\beta_2$-agonists during regular treatment has been demonstrated in other studies [3–5]. It may be that down-regulation of $\beta_2$-receptors in other cells contributed to the overall deterioration in asthma control in our original study.

Down regulation of the $\beta_2$-adrenoceptor in vitro has been shown to be influenced by two common polymorphisms of the receptor gene which change the amino-acid sequence of the N-terminal domain of the receptor [6]. One results in the substitution of glycine (Gly) for arginine (Arg) at amino acid position 16 (Gly 16) of the receptor protein while the other results in the substitution of glutamic acid for glycine at position 16 (Glu 16). In 1990 we reported a randomized controlled trial in which the administration of regular inhaled $\beta_2$-agonist therapy was associated with a deterioration in asthma control in a majority of subjects when compared to as-required $\beta_2$-agonist treatment [1]. There were more asthma exacerbations, reductions in the forced expiratory volume in one second (FEV1) and morning peak expiratory flow rates (PEFRs) and increased bronchial responsiveness to methacholine during regular fenoterol therapy [2].

The mechanism responsible for the deterioration in asthma control during regular $\beta_2$-agonist therapy is unknown, but agonist-induced down-regulation of $\beta_2$-adrenoceptor function is one possible explanation. In our study there was no evidence of loss of the acute bronchodilator response to $\beta_2$-agonist after prolonged therapy, suggesting that down-regulation of airway smooth muscle $\beta_2$-receptors was not a significant problem [2]. However, tolerance to the nonbronchodilating properties of inhaled $\beta_2$-agonists during regular treatment has been demonstrated in other studies [3–5]. It may be that down-regulation of $\beta_2$-receptors in other cells contributed to the overall deterioration in asthma control in our original study.

Down regulation of the $\beta_2$-adrenoceptor in vitro has been shown to be influenced by two common polymorphisms of the receptor gene which change the amino-acid sequence of the N-terminal domain of the receptor [6]. Recent studies have linked these polymorphisms to certain clinical features. Gly 16 has been associated with reduced bronchial responsiveness to methacholine [10]. Recently asthmatic patients homozygous for the Gly 16 polymorphism have been shown to undergo a greater loss of the bronchodilator response to $\beta_2$-agonist during treatment with formoterol. Subjects heterozygous for Gly 16 and Glu 27 polymorphisms down-regulated relatively resistant to down-regulation [7, 8]. Receptors with both Gly 16 and Glu 27 polymorphisms down-regulated to a similar extent to the Gly 16 variant alone [7].

Although these polymorphisms occur with similar frequency in asthmatics and nonasthmatics, among asthmatic patients they have been linked to certain clinical features. Gly 16 has been associated with an increased requirement for oral corticosteroids [6] and nocturnal asthma symptoms [9]. In contrast, Glu 27 has been associated with increased bronchial responsiveness to methacholine [10]. Recently asthmatic patients homozygous for the Gly 16 polymorphism have been shown to undergo a greater loss of the bronchodilator response to $\beta_2$-agonist during treatment with formoterol. Subjects heterozygous for Arg 16/ Gly 16 showed intermediate desensitization [11].

There have been no reports on the influence of $\beta_2$-adrenoceptor polymorphism on changes in asthma control during prolonged $\beta_2$-agonist therapy. We now report an analysis of the $\beta_2$-adrenoceptor genotype in the subjects...
from our previous study [1], in which we have sought to establish whether the change in asthma control during regular β₂-agonist treatment was related to β₂-adrenoceptor polymorphism at amino acids 16 and 27. Our hypothesis was that the polymorphism which gives rise to enhanced down-regulation of the β₂-adrenoceptor (Gly 16) would be associated with a deterioration in asthma control during prolonged β₂-agonist therapy, whereas the polymorphism which is relatively resistant to down-regulation (Glu 27) would be associated with improved or unchanged asthma control.

Methods

Study design

The randomized controlled study of regular versus as-needed β₂-agonist has been reported previously [1]. Briefly, 89 patients with a history of mild to moderate asthma were recruited to a double-blind, randomized, placebo-controlled, cross-over study of treatment with inhaled dry-powder fenoterol (400 µg q.i.d.) or matching placebo for 24 weeks each. Throughout the study, subjects were allowed to use additional known β₂-agonist by metered dose inhaler as required for symptom relief. All subjects had demonstrated bronchial hyperresponsiveness to methacholine (provocative concentration causing a 20% fall in FEV1 (PC20) <8 mg·mL⁻¹) and a significant response to inhaled bronchodilator (>20% rise in FEV1).

Inhaled corticosteroids or cromoglycate were continued providing they had been used at a constant dose for three months before the study. Exacerbations of asthma were treated with increased β₂-agonist and short courses of oral prednisone if needed. All other asthma treatment was withdrawn prior to entry into the study.

Asthma control was monitored from a diary of asthma symptom scores recorded twice daily, morning and evening PEFRs, and additional bronchodilator use. Every 4 weeks, each subject performed spirometry followed by either a methacholine challenge [12] or a bronchodilator response test (repeat spirometry 10 min after nebulized β₂-agonist) at alternate visits. Subjects withheld all bronchodilator medication (including the blinded trial medication) for 6 h before attending the laboratory.

Sixty eight patients completed the study. Of these, four were excluded from analysis because of protocol violations [1]. Data from 64 subjects were analysed.

The period of better asthma control was judged by within-subject comparisons of data from weeks 9–24 of each treatment period using predefined criteria: the need for short courses of prednisone, morning PEFR, additional nocturnal bronchodilator use, nocturnal symptoms, daytime symptoms, evening PEFR and additional daytime bronchodilator use.

Further analyses compared the effects of regular β₂-agonist with placebo on lung function, methacholine responsiveness, bronchodilator responsiveness, morning and evening PEFR, diurnal PEFR variation and asthma exacerbations [2].

Identification of polymorphisms

All 64 subjects who had satisfactorily completed the trial were invited to provide venous blood samples for the identification of their β₂-receptor genotype. Two were living overseas and unable to participate and one refused. Samples were obtained from the remaining 61 subjects. Informed consent was obtained from all participants. The study was approved by the Southern Regional Health Ethics Committee (Otago).

Deoxyribonucleic acid (DNA) was extracted from the citrated blood sample using a commercial kit (QIAamp; Qiagen, Hilden, Germany) within 48 h of obtaining the sample. DNA specimens were kept frozen until transfer to the University of Cincinnati for genotyping. Identification of the polymorphisms at nucleic acids 46 and 79 of the β₂-receptor gene (coding for the amino acids at position 16 and 27 of the β₂-receptor protein) was performed using allele-specific polymerase chain reaction as previously described [9].

Statistical analysis

The frequencies of the different polymorphisms of the group judged to have had an overall improvement in asthma control during regular fenoterol treatment in the earlier study were compared with the frequencies for the group whose asthma deteriorated and the group whose asthma showed no change during regular treatment (Chi-squared). In addition, the changes in morning PEFR, evening PEFR, diurnal variation of PEFR, daytime and night-time asthma symptoms, additional bronchodilator use, FEV1, methacholine responsiveness and bronchodilator response that occurred between the regular compared to the as-needed treatment periods were calculated for each of the genotypes at position 16 and 27. Differences in the between-treatment changes were compared using a repeated-measures analysis of variance (MANOVA) (Statistical Products and Service Solutions; SPSS Inc., Chicago, IL, USA).

Results

The genotype for position 16 was determined in all 61 subjects. The allelic frequency (percentage of the total 122 alleles) of Gly 16 was 66% (49% of subjects were homozygous for Gly 16). The genotype for position 27 could

<table>
<thead>
<tr>
<th>Position 27</th>
<th>Position 16</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly/Gly</td>
<td>Gly/Arg</td>
<td>Arg/Arg</td>
</tr>
<tr>
<td>Gly/Glu</td>
<td>17</td>
<td>1</td>
</tr>
<tr>
<td>Gly/Gln</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>29</td>
<td>19</td>
</tr>
</tbody>
</table>

The genotype for position 27 could not be identified in one subject (not included in table). For position 16 the "wild-type" is arginine (Arg). For position 27 the "wild-type" is glutamine (Gln). Chi-squared is 45.7 with 4 degrees of freedom (p<0.0001). Gly: glycine; Glu: glutamic acid.
not be determined in one subject. In the remainder the allelic frequency of the Glu 27 polymorphism was 51% (30% homozygous). There was a strong linkage between the polymorphisms (table 1) (Chi-squared, p<0.0001).

The genotype of those whose asthma was better controlled during the 24 weeks in which they used regular β-agonist therapy (n=16) did not differ significantly at either position 16 or 27 from those whose asthma showed no change (n=7) or deteriorated (n=38) during the regular treatment period (table 2).

When the responses to treatment were compared between genotypes, significant differences were found for two outcome variables (table 3):

1) The change in bronchial responsiveness to methacholine during regular compared to as-required β-agonist treatment was significantly different between genotypes (p=0.029). Subjects homozygous for Gly 16 did not show a deterioration in bronchial responsiveness during regular treatment (+0.08 doubling dose shift in PC20), whereas heterozygotes and homozygotes for Arg 16 did (-0.71 and -0.88 doubling dose shifts, respectively). Change in methacholine responsiveness during treatment was not associated with polymorphism at position 27.

2) Subjects homozygous or heterozygous for Gln 27 had higher evening PEFRs during regular fenoterol treatment (attributable to the measurements being made 1–2 h after the evening dose of fenoterol). Subjects homozygous for Glu 27 did not show this increase in evening PEFR during regular treatment but showed a decrease in evening PEFR change between groups. Change in evening PEFR was not affected by the polymorphisms at position 16.

No relationships were found between the genotypes at either position 16 or 27 and between-treatment changes in any of the following measures: morning PEFR; diurnal variation in PEFR; FEV1; additional bronchodilator use; bronchodilator response; or asthma symptom scores (table 3).

Table 2.—Treatment period associated with best overall asthma control by genotype

<table>
<thead>
<tr>
<th>Best treatment period</th>
<th>Position 16 (n=61)</th>
<th>Position 27 (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arg/Arg</td>
<td>Arg/Gly</td>
</tr>
<tr>
<td>Regular</td>
<td>Arg/Arg</td>
<td>Arg/Gly</td>
</tr>
<tr>
<td>As required</td>
<td>2 (17)</td>
<td>4 (21)</td>
</tr>
<tr>
<td>No difference</td>
<td>3 (25)</td>
<td>3 (16)</td>
</tr>
<tr>
<td>As required</td>
<td>7 (58)</td>
<td>12 (63)</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>19</td>
</tr>
</tbody>
</table>

Values are presented as the number of subjects, and percentage in parenthesis, with each genotype whose asthma control was better during regular or as required β-agonist treatment or in whom there was no difference between treatment periods. For definitions see legend to table 1.

Table 3.—Mean values during as-required and regular beta-agonist treatment for each of the genotypes controlling for amino acid residues 16 and 27, and for all subjects

<table>
<thead>
<tr>
<th>Best treatment period</th>
<th>Position 16 (n=61)</th>
<th>Position 27 (n=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arg/Arg</td>
<td>Arg/Gly</td>
</tr>
<tr>
<td>FEVI L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>As req. (n=12)</td>
<td>2.44</td>
<td>415</td>
</tr>
<tr>
<td>Reg.</td>
<td>2.22</td>
<td>405</td>
</tr>
<tr>
<td>Δ</td>
<td>-0.22</td>
<td>-10</td>
</tr>
<tr>
<td>As req. (n=19)</td>
<td>2.55</td>
<td>396</td>
</tr>
<tr>
<td>Reg.</td>
<td>2.48</td>
<td>391</td>
</tr>
<tr>
<td>Δ</td>
<td>-0.07</td>
<td>-5</td>
</tr>
<tr>
<td>As req. (n=30)</td>
<td>2.26</td>
<td>386</td>
</tr>
<tr>
<td>Δ</td>
<td>-0.18</td>
<td>-10</td>
</tr>
</tbody>
</table>

The values are presented as overall means during each treatment period. ¹: geometric mean values, difference expressed as doubling dose shifts; ²: percentage improvement in forced expiratory volume in one second (FEVI) following bronchodilator; ³: p=0.029, comparing Gly/Gly with Arg/Arg and Arg/Gly; ⁴: p=0.037, comparing Glu/Glu with Gln/Gln and Gln/Glu. PEFR: peak expiratory flow rate; PC20: provocative concentration of methacholine causing a 20% fall in FEV1; BDR: bronchodilator response; Extra BD: mean bronchodilator use during treatment period; Arg: arginine; Gly: glycine; Glu: glutamic acid; Gln: glutamine; As req.: as required; Reg.: regular; Δ: difference.
Because of the strong linkage between the genotypes (see table 1) it was not possible to assess the effects of different combinations of the 16 and 27 polymorphisms or to separate the independent effects of each polymorphism. However, it was possible to compare subjects who were heterozygous for Gln 27 but homozygous for Gly 16 (n=10) with those who were heterozygous for both Gln 27 and Gly 16 (n=14) (i.e. differing only at position 16). In this comparison, those who were homozygous for Gly 16 did not experience an increase in bronchial responsiveness to methacholine during regular treatment, whereas those who were heterozygous did (0.35 and -0.91 doubling dose shifts, respectively, p=0.008). No other significant differences were found. Comparison was also made between subjects who were homozygous for both Gly 16 and Gln 27 (n=17) and those who were homozygous for Gly 16 but heterozygous for Gln 27 (n=10) (i.e. differing only at position 27). No significant differences occurred between these groups for any outcome variable.

To establish whether the simultaneous inheritance of both polymorphisms altered to response in β2-agonist therapy, a comparison was made between the 17 subjects homozygous for both Gly 16 and Gln 27 and the 11 subjects homozygous for Arg 16 and Gln 27 (i.e. differing at both the 16 and 27 position). Subjects with the Gly 16/ Gln 27 combination had a smaller increase in diurnal PEFR variation during active treatment than subjects with Arg 16/ Gln 27 (3.9 and 10.9%, respectively, p=0.045). No other significant differences were identified.

**Discussion**

This study did not find an association between β2-adrenoceptor genotype and change in asthma control during regular high dose inhaled β2-agonist therapy. We had hypothesized that the β2-receptor polymorphism associated with enhanced receptor down-regulation in vitro (Gly 16) would be associated with enhanced receptor down-regulation to methacholine during regular treatment, whereas those who were heterozygous did (0.35 and -0.91 doubling dose shifts, respectively, p=0.008).

In this study, the group who were heterozygous at position 27 it appeared that the Gly 16 polymorphism protected against deterioration in bronchial responsiveness during regular β2-agonist treatment, thus confirming one of the findings from the whole group. No other significant association was identified. In vitro the simultaneous expression of both polymorphisms results in enhanced down-regulation to a similar extent to the Gly 16 polymorphism alone [7]. Whether the interaction between these polymorphisms is different in vivo is unknown. In this study, the group who were homozygous for both polymorphisms (Gly 16 and Gln 27) had a smaller increase in diurnal peak flow variation during regular β2-agonist treatment compared to those with neither polymorphism, but there were no other differences.

The role of β2-receptor regulation in asthma is unclear. While tachyphylaxis to the bronchodilator effects of β2-agonists occurs in nonasthmatic individuals, it has been difficult to demonstrate in asthmatics [13–17]. However, tolerance to the bronchoprotective effects of β2-agonists in asthma has been demonstrated [3–5]. Thus, the mechanisms of β2-receptor regulation in asthma appear to differ between cell types and may be altered by disease states [18]. It has recently been reported that asthmatic subjects homozygous for Gly 16 demonstrate greater tachyphylaxis to the long-acting β2-agonist formoterol [11]. In the present study we found no difference in bronchodilator tachyphylaxis between genotypes. Against this background, the clinical importance of in vitro differences in regulation of β2-receptors conferred by polymorphisms of the receptor protein is difficult to interpret.
In conclusion, polymorphism of the β₂-receptor in 61 New Zealand asthmatics did not appear to determine the response to long-term inhaled β₂-agonist treatment. Subjects of all genotypes demonstrated poorer control of asthma when treated with regular fenoterol compared with as-required therapy. The apparent protective effect of the Gly 16 polymorphism against deterioration in bronchial responsiveness with regular β₂-agonist treatment warrants further investigation. The mechanism underlying the overall deterioration in asthma control during regular β₂-agonist treatment is still to be explained.

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References